Determinants of bleeding in obstetrics and gynaecology

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General introduction and outline of thesis

Part I Menorrhagia and bleeding disorders

General introduction

Menorrhagia is a common problem among women. At least 5-10% of women in reproductive age seek medical attention for menorrhagia.[1] The World Health Organization estimates that 18 million women worldwide are affected.[2] Menorrhagia is objectively defined as greater than or equal to 80 ml blood loss per menstrual cycle[3] and is a common cause of iron deficiency anaemia.[3] This can affect a woman's quality of life, her study or work and family and social interactions.[4] Historically, the causes of menorrhagia have focused on gynaecological and endocrinological conditions in terms of organic pathology (e.g. polyps and fibroids) and anovulation/ hormonal dysbalance. After excluding these conditions, the remaining aetiologies were systemic disorders such as hypothyroidism[5] and iatrogenic causes including intrauterine devices and the use of anticoagulants.[1] In the past, prior to broad haemostatic testing in patients with menorrhagia, no specific aetiology was identified in approximately 50% of cases, leading to the diagnosis of exclusion 'dysfunctional uterine bleeding'.[6] Normal menstruation can be described in part as a haemostatic process given the necessity for monthly formation of a primary platelet aggregate with subsequent secondary fibrin formation with concurrent fibrinolytic modelling of the fibrin clot. For women with menorrhagia without gynaecological abnormalities, haemostatic evaluation for underlying bleeding disorders,[7-9] including von Willebrand`s disease (VWD), platelet dysfunction and coagulation factor deficiencies has been advised.[7, 8, 10] The prevalence of underlying bleeding disorders in women with menorrhagia varies from 1 to 47%.[11,12] The highest prevalence is reported for platelet dysfunction, but this is based on one single study[12] and is yet not well established. For VWD the prevalence varies from 5 to 24% with an overall prevalence of 13% (95% confidence interval, 11-16%) based on a systematic review of 11 studies comprising 988 women with menorrhagia.[11] Furthermore, it is important to realize that most studies measured randomly in the menstrual cycle. This could give an over- or underestimation of the prevalence of bleeding disorders,[11] because a cyclic variation of haemostatic variables with the lowest levels during menstrual and/or follicular phase have been found.[13] Previous studies have focussed on women without gynaecological abnormalities. Whether women with menorrhagia and gynaecological abnormalities as uterine polyps and fibroids also have underlying bleeding disorders has not been assessed in earlier studies.

Haemostatic variables during menstrual cycle

A wide range of values for coagulation and clotting factors has been reported in both normal individuals and patients with bleeding disorders, with a considerable overlap between normal subjects and patients with mild disorders, especially in patients with VWD. In addition, a wide inter-individual and intra-individual variability of these coagulation and clotting factors has been reported. This variability could be the effect of hormonal changes. Estradiol concentrations are lowest on cycle

days (cd) 1-3 and highest on cd 13-15, followed by a decrease. Progesterone concentrations are lowest on cd 1-8 and highest on cd 21-25. Such hormonal variation could have important implications for the timing of haemostatic evaluation in women with menorrhagia. For that reason, we decided to summarize the evidence for the timing of haemostatic evaluation in women with menorrhagia.

Women with von Willebrand`s Disease

Theoretically, men and women are equally likely to be affected with VWD, but in women the disorder is more often clinically manifest because of the bleeding challenges that are associated with menstruation and childbirth.[14] Most of the studies performed so far addressed these bleeding problems only in mild type 1 disease rather than the more severe VWD types, and most of these studies are small case series. In these studies women with VWD frequently have menorrhagia, with reported prevalence ranging from 74 to 92%.[15-17] This may lead to impaired quality of life. Several medical and surgical treatment options are available for menorrhagia, and the appropriate choice of therapy can be tailored to the individual needs of the patient. Nevertheless, women with VWD are more likely to undergo a hysterectomy and they undergo this operation at a younger age,[18] despite the increased risk of bleeding complications. The above mentioned studies may suffer from selection bias given the fact that patients seeking medical attention for bleeding and menorrhagia have predominantly been included. Therefore, we initiated an investigation on the gynaecological and obstetrical symptoms in a large unselected cohort of women who participated in the WiN-study, a nation-wide study on moderate and severe VWD in the Netherlands.

Part II Bleeding issues in obstetrics

General introduction

Low-molecular-weight heparins (LMWH) are the most commonly used anticoagulant during pregnancy for prevention or treatment of venous thromboembolism (VTE). Pulmonary embolism (PE) is the second cause of maternal mortality in the Western world, and deep vein thrombosis (DVT) in pregnancy is an important cause of maternal morbidity, also on the long term.[19,20] Venous thromboembolism complicates 1 to 2 of 1000 pregnancies, and the risk increases with age, mode of delivery, and presence of comorbidities.[19, 21, 22] In women with a previous episode of VTE, the risk of recurrence during pregnancy ranges from 2-6%.[23,24] For these women, with either a current VTE or a high risk of recurrent VTE, LMWH is the most commonly used anticoagulant during pregnancy for prevention of a new thrombotic event.

The usage of LMWH during pregnancy

The usage of LMWH during pregnancy may be associated with an increased risk of blood loss or postpartum haemorrhage (PPH), a common complication of childbirth and one of the causes of maternal morbidity and mortality. Few studies assessed the risk of PPH associated with usage of LMWH,[25-32] but most studies are retrospective cohort studies, without a control group and describing only a small number of women on therapeutic dosage LMWH.

Moreover, the optimal dosage of thromboprophylaxis in women with an increased risk of VTE during pregnancy and puerperium is not well established.[33] Prophylactic, intermediate as well as therapeutic doses of LMWH are used.[34, 35] Further, current guidelines recommend discontinuing LMWH at least 24 hours before labor,[33] although no data are available whether this decreases the risk of PPH. In our hospital, all pregnant women with an indication for thromboprophylaxis received a therapeutic dosage of LMWH during pregnancy and the puerperium. We could therefore assess a relatively large cohort of pregnant women on full dose LMWH. First, we evaluate the risk of PPH in women who used a therapeutic dosage of LMWH. Second, we assess the bleeding risk in relation to the last injection of LMWH. Another well-known, but underreported complication of the usage of LMWH are hypersensitivity skin reactions. This could be an intolerance for heparin, but pregnancy seems to increase the incidence of these skin reactions. However, alternative choices for anticoagulation are limited and hypersensitivity skin reactions might recur when another preparation of LMWH is used.[36] A few studies reported on the incidence of hypersensitivity skin reactions on LMWH in pregnant women, but these studies had other primary outcomes and therefore hypersensitivity skin reactions are probably underreported.[37-39] To evaluate the incidence of hypersensitivity skin reactions, we used the same cohort as for the bleeding risk.

Alternative anticoagulation is limited, but fondaparinux, a synthetic selective inhibitor of activated factor X (fXa), is commonly used as an alternative anticoagulant in non-pregnant patients who develop

heparin intolerance. However, data on the use of fondaparinux in pregnancy is limited to animal models and a few case reports.[42-47] While we are collecting data about the usage of LMWH during pregnancy, we observed during clinical practice that some women had hypersensitivity skin reactions to all preparations of LMWH. And most patients strongly wish to avoid vitamin K antagonists, even beyond the 12th week of pregnancy, because of the association with congenital and developmental abnormalities.[40, 41] They used fondaparinux as an alternative anticoagulation. Because of the limited data in the literature, we described our experience of these 10 women.

Reproductive choices and obstetrical experience in Dutch carriers of haemophilia A and B

Haemophilia is an X-linked recessive inherited bleeding disorder that arises from reduced levels of functional coagulation factor VIII (haemophilia A) or factor IX (haemophilia B). Males inherit the condition whilst females are affected as carriers. Carriers of haemophilia are expected to have clotting factor levels around 50% of normal as they have only one affected chromosome. However, a wide range of values (0.05–2.19 IU mL)[42] has been reported as a result of lyonization, e.g. random inactivation of one of both X chromosomes.[43] Some haemophilia carriers may have very low factor levels and therefore have an increased tendency to bleed.[42]

In the Dutch obstetrical care system, women with low-risk pregnancies can choose between home and outpatient hospital delivery. About 30% of the deliveries take place at home.[44] This means that in the past, and also presently in women with unknown carrier state of haemophilia, women with unknown levels of plasma factor could be at risk of bleeding during pregnancy and delivery in a low-tech environment. From 2000 onwards, national guidelines advise to refer all known carriers, irrespectively of factor levels and fetal sex, to the obstetric department of a hospital with a Haemophilia Centre. Despite advances in care, pregnancy and delivery seems to remain critical times for carriers of haemophilia, with previous studies reporting 9–38% bleeding complications.[45-48] Therefore, we want to evaluate our experience with pregnancy and delivery in carriers of haemophilia during the last decennia and after the introduction of the national guideline in the Netherlands. Secondly, we evaluate the influence of the knowledge of carriership of haemophilia on reproductive choices.

Outline of this thesis

The first part of this thesis focuses on diverse aspects of haemostatic testing and clinical symptoms of women with menorrhagia. Menorrhagia can be associated with a wide range of haemostatic disorders**.** In **Chapter 1** we summarize the evidence for timing of haemostatic testing during the menstrual cycle in women with a suspected bleeding disorder. **Chapter 2** investigates the work-up of menorrhagia in the University Medical Centre of Groningen, with a special interest in haemostatic evaluation. Secondly, the outcome of routine treatment in patients with unexplained menorrhagia is assessed. Because haemostatic testing was not routinely done in women with menorrhagia, we hypothesized that the identification of haemostatic disorders might improve care for these women. Therefore, we designed in **Chapter 3** a prospective cohort study of women with menorrhagia to evaluate the prevalence of underlying bleeding disorders, including VWD, other coagulation disorders and platelet defects, in patients with unexplained and explained menorrhagia with testing in the 1st week after menstruation. Women with von Willebrand`s disease have frequently bleeding episodes during menstruation and childbirth. To investigate this we assess in **Chapter 4** the gynaecological and obstetrical symptoms in a large unselected cohort of women with moderate or severe VWD who participated in the WiN-study, a nation-wide cross-sectional study among patients with moderate and severe VWD in the Netherlands

The second part of this thesis focuses on different bleeding issues in the obstetrical practice. Low-molecular-weight heparins are the most commonly used anticoagulation drugs during pregnancy and puerperium for treatment and prevention of venous thrombo-embolism. This could be complicated by an increased bleeding risk and hypersensitivity skin reactions due to the daily injections. **Chapter 5** assesses the bleeding risk of the usage of a therapeutic dosage low-molecular-weight heparins during pregnancy and delivery compared to controls. Second, this chapter assesses the bleeding risk in relation to the last injection of LMWH. **Chapter 6** assesses the prevalence of hypersensitivity skin reactions of LMWH usage during pregnancy. When these skin reactions occur, alternative anticoagulation are limited. **Chapter 7** evaluates the usage and safety of fondaparinux as an alternative anticoagulation during pregnancy. **Chapter 8** evaluates the reproductive choices and obstetrical experiences in the current generation of carriers of haemophilia in our Haemophilia Centre. **Chapter 9** addresses the risk of recurrent fetal loss in women with thrombophilic defects and high levels of TAFI.

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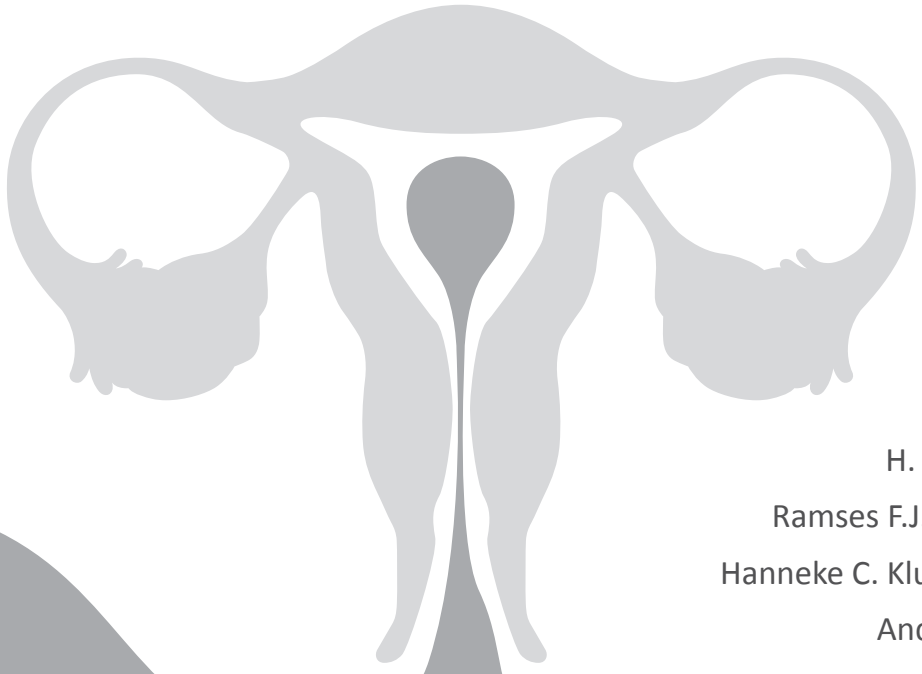
Part I

Menorrhagia and bleeding disorders

Chapter 1

Haemostatic variables during normal menstrual cycle, a systematic review



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Abstract

Background: for a number of haemostatic factors menstrual cycle variation has been studied. Such variation could have clinical implications for the timing of haemostatic testing in women.
Objectives: systematically review the literature about evidence for timing of haemostatic testing during menstrual cycle.

Methods: we searched MEDLINE, EMBASE and the Cochrane library to identify studies that measured haemostatic variables (platelet function, von Willebrand factor, factor VIII, factor IX, factor XI, factor XIII, d-dimer, PAI-I, tPA, alpha-2-antiplasmin and fibrinogen during normal menstrual cycle without hormonal contraceptives. Two investigators independently selected studies, and abstracted data in duplicate.

Results: we identified 1046 studies of which we included 30 studies (25 longitudinal and 5 cross-sectional studies). All studies reported on haemostatic variables during menstrual cycle. Overall, most of the studies found no cyclic variation in von Willebrand factor, FVIII, FXI, FXIII, fibrinolytic factors (PAI, t-PA, uPA, d-dimer and α2-antiplasmin) and fibrinogen. However, in studies where any these variables showed any variation, they reached the lowest levels during menstrual and early follicular phase, especially for von Willebrand Factor, FVIII and platelet function tests.

Conclusion: the optimal timing for haemostatic testing during menstrual cycle seems to be menstrual and early follicular phase.

Background

Menorrhagia is a very common clinical condition affecting about 5% of women. For women with menorrhagia without gynaecological abnormalities haemostatic evaluation for underlying bleeding disorders,[1,2] including von Willebrand`s disease (VWD), platelet dysfunction and coagulation factor deficiencies has been advised.[3] The prevalence of underlying bleeding disorders in women with menorrhagia varies from 1 to 47%.[4] The highest prevalence is reported for platelet dysfunction, but this is based on one study.[5] For VWD the prevalence varies from 5 to 24% with an overall prevalence of 13% (95% confidence interval, 11-16%) based on a systematic review of 11 studies comprising 988 women with menorrhagia.[6]

Interestingly, a wide range of values for coagulation and clotting factors has been reported in both normal individuals and patients with bleeding disorders, with a considerable overlap between normal subjects and patients with mild disorders, especially in patients with von Willebrand`s disease. In addition, a wide inter-individual and intra-individual variability of these coagulation and clotting factors has been reported. This variability could be the effect of hormonal changes. Estradiol concentrations are lowest on cycle days (cd) 1-3 and highest on cd 13-15, followed by a decrease. Progesterone concentrations are lowest on cd 1-8 and highest on cd 21-25. Menstrual cyclic variation has been studied for a number of haemostatic factors. Such variation could have important implications for the timing of haemostatic evaluation in women with menorrhagia. The aim of this systematic review is to summarise the evidence for timing of haemostatic testing during the menstrual cycle in women with a suspected bleeding disorder.

Methods

Search strategy and study selection

We identified haemostatic variables relevant to this review from previous knowledge and a non-systematic review of the literature. Subsequently, we searched the MEDLINE database using the terms 'menstrual cycle', 'menstruation', 'menorrhagia', 'blood coagulation factors', 'aPTT', 'PT', 'blood platelet disorders', 'von Willebrand factor', 'factor VIII', 'factor IX', 'factor XI', 'factor XIII', 'd-dimer', 'fibrinolysis', 'PAI', 'tPA', 'plasmin inhibitor', 'alpha-2-antiplasmin' and 'fibrinogen' as MeSH terms and as text words. A similar search was performed in the EMBASE database and the Cochrane Database of Systematic Reviews with the same terms. The reference lists of relevant articles were searched for additional studies. The literature search was performed in August 2010. After identifying relevant titles, abstracts of these studies were read to decide if the study was eligible. The full article was retrieved when the information in the title or abstract appeared to meet the inclusion criteria of this systematic review. All materials were reviewed independently by two investigators (HMK and RFJK).

We included all study designs (cross-sectional and longitudinal studies), if they included women with a normal regular menstrual cycle and/or women with menorrhagia, who did not use oral contraceptives, in whom haemostatic variables were tested during the menstrual cycle at two or more

moments. We excluded studies if they included less than 5 women. No language restrictions were applied; all papers included in the final selection were in English. We applied no restriction in time. Disagreements between the two primary reviewers were resolved by discussion.

Analysis

We present study characteristics and the days of sampling in different tables per (group of) haemostatic variable(s). A priori, we determined that if there was significant clinical, methodological or statistical heterogeneity among included studies we would not pool data. Although we did not measure heterogeneity quantitatively, the design, case mix and the different days of sampling haemostatic variables in the included studies were, as expected, so heterogeneous that statistical pooling of results was not justified. We fitted a figure with the vWF levels during the follicular (cd 5-9) and the luteal phase (cd 19-23) and with fibrinogen levels during follicular (cd 5-11) and luteal phase (cd 17-28).

Results

A total of 1048 studies were identified with our search (figure 1). We excluded 998 studies after screening titles and abstracts by using the predefined inclusion and exclusion criteria. Forty-eight studies underwent full text review, 18 studies were excluded for the following reasons: measured haemostatic variables in all patients on only one moment during menstrual cycle (n=5), number of patients < 5 (n=1), did not address the question of interest (n=8) and had duplicate data (n=1). A search of the reference lists, EMBASE and Cochrane databases did not yield any more relevant articles. Therefore, we included 30 studies for this review.

Platelets

We identified two studies that tested platelet function by platelet function analyzer (PFA) and one study that performed a thrombocytogram during the menstrual cycle. See also table 1.

We found two longitudinal studies[7,8] and one cross-sectional study.[9] Both longitudinal studies sampled during follicular and luteal phase in the menstrual cycle. Roell et al.[9] performed a cross-sectional study and compared the platelet function of women in the follicular phase (n=27) and luteal phase (n=25). All studies established the menstrual cycle to be ovulatory by measuring hormones. The individual sample size varied from 10 to 52 healthy volunteers. Feuring et al.[7] and Roell et al.[9] performed platelet function analyses by PFA-100 using collagen/epinephrine-coated and collagen/ADP-coated cartridges. Both studies found an increased platelet function during luteal phase. Roell et al. al.[9] also found an increase of vWF-levels in response to increasing progesterone levels during the luteal phase of the menstrual cycle. Repina et al.[8] performed a longitudinal study of aggregation tests with ADP, ristocetin and collagen during the follicular and luteal phase in 10 pre- and perimenopausal women and found no cyclic variation.

Figure 1: Study flow diagram

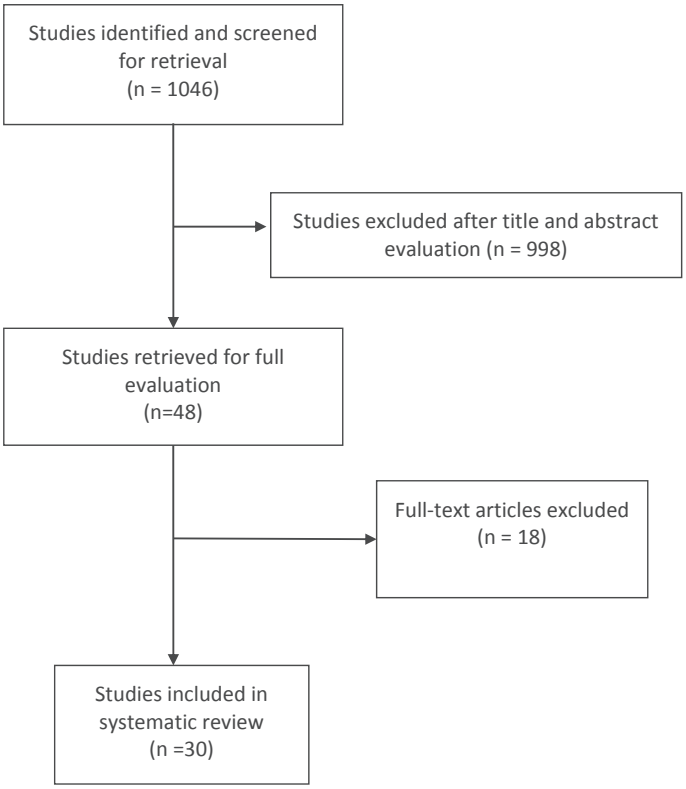


Table 1: Platelet function during the menstrual cycle

Study	Number of patients	Laboratory measurements	Hormone analysis Y/ N	Levels lowest during (cd or phase)	Measurement (cd or phase)	
					1	2
Roell et al, 2007	52	PFA-100	Y	F	Mid F	Mid L
Feuring et al, 2002	18	PFA-100	Y	8-11	8-11	18-21
Repina et al, 2002	10	aggregation	Y	NA	F	L

NA= not applicable (no cyclic variation) Y= yes; N= no; cd= cycle days
F= follicular phase; L= luteal phase PFA= platelet function analyzer

Von Willebrand factor

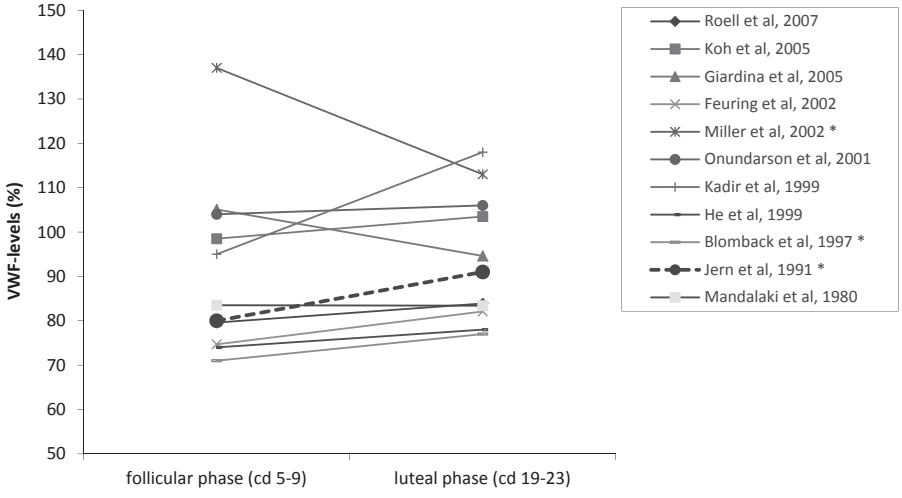
Eleven studies were identified that tested vWF levels during the menstrual cycle, varying from two to sixteen times. The characteristics of the studies are presented in table 2.

We found 7 longitudinal[7, 10-15] and 2 cross-sectional studies.[9,16] One study had 2 designs, namely

a longitudinal and a cross-sectional part.[17] The longitudinal studies measured vWF-levels on at least two moments during the follicular and luteal phase, but four studies measured it on more moments, varying from 3 to 6 times. In most of the studies the days of sampling were different. The cross-sectional studies tested women on one moment and compared the levels by grouping the women in groups by time of sampling during the menstrual cycle. Individual study sizes ranged from 9 to 123 women. All studies included healthy volunteers, except one study by Kadir et al., who performed a cross-sectional analysis in 123 women with menorrhagia.[17] In total, 7 studies[7, 9-13,15] established an ovulatory menstrual cycle by measuring hormones.

Overall, five out of eleven studies found a cyclic variation. All these five studies[9, 11, 13, 16, 17] reported the lowest vWF levels during menstruation or early follicular phase (cd 1-7). One of these studies by Kadir et al.[17] found in their longitudinal data of 19 normal menstruating volunteers a statistically significant decrease of vWF-Ag and vWF-Rcf concentrations during the first 3 days of the menstruation. In one study by Roell et al.[9] the exact days of sampling are lacking, but they reported the lowest levels mid follicular. VWF-levels were mostly about 10% (range 2-24%) lower in menstrual/ early follicular phase compared to the luteal phase (see figure 2). The other six studies reported no cyclic variation.[7, 10, 12, 14, 15, 18]

Figure 2: Von Willebrand factor (VWF) (%) levels during the follicular and luteal phase



* = significant variation of vWF-levels

Table 2: Von Willebrand factor and factor VIII during the menstrual cycle

Study	Number of patients	Haemostatic factors	Hormone analysis Y/N	Levels lowest in (cd or phase)	Measurement (cd or phase)					
					1	2	3	4	5	6
Roell et al, 2007	52	vWF-ag	Y	mid F	mid F	mid L				
Koh et al, 2005	30	FVIII, vWF	N	NA	1-3	5-9	10-14	21-26		
Giardina et al, 2005	20	vWF-ag	Y	NA	F	L				
Repina et al, 2002	10	FVIII	Y	NA	F	L				
Feuring et al, 2002	18	vWF	Y	NA	8-11	18-21				
Miller et al, 2002	90	FVIII; vWF-ag, vWF-RCF	N	1-4	1-4	5-7	8-11	12-16	17-21	22-30
Onundarson et al, 2001	95	vWF-ag, vWF-RCF, FVIII	Y	NA	4-7	11-15	21-28			
Kadir et al, 1999	142 †	vWF-ag, vWF-RCF, FVIII	N	1-3 (VWF-ag/RCF)	1-4	6-11	10-18	20-25		
He et al, 1999	24	vWF-ag	N	NA	5-7	24-26				
Blomback et al, 1997	15	vWF-ag, FVIII	Y	5-7 (vWF-ag)	5-7	20-25				
Blomback et al, 1992	10	vWF-ag, FVIII	Y	NA	unknown					
Jern et al, 1991	9	vWF-ag	Y	NA	2-8	18-26				
Siegbahn et al 1989	13	FVIII	Y	NA	early F	late F	early L	late L	M	
Mandalaki et al, 1980	9	FVIII:C; FVIII:ag; VIII:RCF	N	5-7	5-7	14	20	1		

F= follicular phase; O= ovulation; L= lutheal phase; M= menstruation; cd=cycle days; NA= not applicable (no cyclic variation) † = 123 of them were menorrhagia patients
Y=yes; N=no vWF= von Willebrand factor ag= antigen RCF= ristocetin cofactor

Factor VIII

We identified 9 studies that tested FVIII levels during menstrual cycle. Study characteristics are presented in table 2.

We found 7 longitudinal studies,[8, 10, 11, 14, 15, 19, 20] 1 cross-sectional[16] and 1 combined study with a longitudinal and a cross-sectional part.[17] Individual study sizes ranged from 9 to 123 women. The longitudinal studies measured FVIII two to six times during the menstrual cycle. In the cross-sectional study by Miller et al.[16] women were tested on one moment and FVIII levels were compared by grouping the women in 6 groups by time of sampling during the menstrual cycle. The phase of the menstrual cycle was estimated for each subject by subtracting the date of the last menstrual period from date of blood collection. In total, 5 studies established an ovulatory menstrual cycle by measuring hormones.[8, 10, 11, 15, 20] Two out of nine studies[16, 19] reported a cyclic variation of FVIII during menstrual cycle. The lowest levels were found during menstruation and early follicular phase. All other studies reported no cyclic variation.[8, 10, 11, 14, 15, 17, 20]

Factor IX

We found no studies that had tested cyclic variation of FIX during menstrual cycle.

Factor XI

Only Kadir et al.[17] tested FXI levels during the menstrual cycle and found no cyclic variations of FXI levels. See table 3.

Factor XIII

We found one longitudinal study by Bolis et al.,[21] which tested cyclic variation of FXIII during the menstrual cycle. They tested 10 healthy volunteers on 4 moments during the menstrual cycle. They found a highly significant decrease during the peri-ovulatory phase of FXIII. See table 3.

Table 3: Other haemostatic variables during the menstrual cycle

Study	Number of patients	Factors	Hormone analysis Y/N	Levels lowest in (cd)	Measurement (cd)			
					1	2	3	4
Kadir et al, 1999	142 †	FXI	N	NA	1-4	6-11	10-18	20-25
Bolis et al, 1982	10	FXIII	N	13-15	6-10	13-15	20-23	27-29

NA= not applicable (no cyclic variation); cd=cycle days † = 123 of them were menorrhagia patients Y= yes; N= no

Fibrinolysis

We identified 15 studies that tested fibrinolytic parameters as plasmin activator inhibitor (PAI-I), tissue plasmin activator (t-PA), d-dimer and α2-antiplasmin during the menstrual cycle. PAI-I is the

principal inhibitor of t-PA and urokinase (uPA), the activators of plasminogen and hence fibrinolysis. See table 4 for detailed information about these studies.

Table 4: Fibrinolytic parameters during the menstrual cycle

Study	Number of patients	Haemostatic factors	Hormone analysis Y/N	Levels lowest in (cd or phase)	Measurements (cd or phase)				
					1	2	3	4	5
Toth et al, 2007	27	d-dimer	Y	NA	F	L			
Koh et al, 2005	30	d-dimer, t-PA, u-PA, PAI-I (ag+act)	N	10-14 (d-dimer)	1-3	5-9	10-14	21-26	
Giardina et al, 2005	20	d-dimer, t-PA-ag, PAI-I-act	Y	L (d-dimer and PAI-I)	14	28			
Ricci et al, 2004	41	t-PA-ag, PAI-I-act	Y	-7 (t-PA)	1	7	14	21	
Feuring et al, 2002	18	d-dimer	Y	NA	8-11	18-21			
He et al, 1999	23	t-PA-ag, PAI-act	N	NA	5-7	24-26			
Chung et al, 1998	18	PAI-I, uPA	Y	F (PaI-I)/ O (uPA)	F	O	L		
Spona et al, 1997	20	d-dimer, t-PA, PAI	N	NA	7	14	21		
Blomback et al, 1997	15	PAI-I, t-PA	Y	NA	5-7	20-25			
Larsen et al, 1996	10	PAI, t-PA	Y	NA	Twice weekly samples during 9 weeks				
Dorr et al, 1993	19	PAI-I-ag, t-PA-Ag; t-PA-act, uPA-ag	Y	19-22 (uPA-ag)	9-12	19-22			
Jern et al, 1991	9	t-PA (act +ag), PAI-I-act	Y	NA	2-8	18-26			
Siegbahn et al, 1989	13	PAI-I	Y	NA	early F	late F	Early L	late L	M
Jespersen et al, 1986	15	t-PA(act+I)	Y	NA	1-3	5-7	12-16	19-23	27-30
Jepsersen et al, 1983	15	α2-antiplasmin-activity	N	12- 16 (α2-antiplasmin-act)	1-3	5-7	12-16	19-23	27-23

F= follicular phase; O= ovulation; L= luteal phase; M= menstruation; cd= cycle days; NA= not applicable (no cyclic variation) Y=yes; N=no; t-PA= tissue-plasmin activator; PAI-I=plasmin activator inhibitor; uPA= urokinase plasmin activator; Act= activity; ag= antigen; I=inhibitor

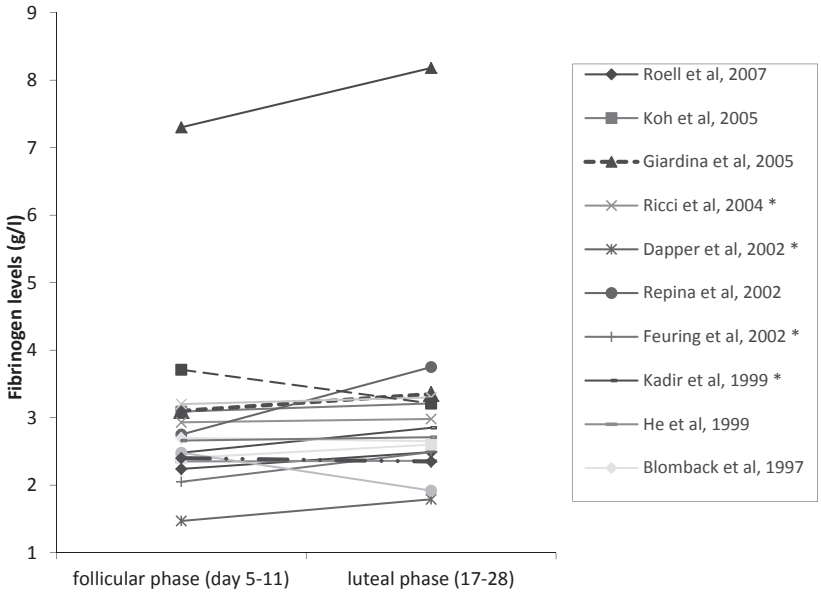
We found 13 longitudinal[7, 11-14, 18, 20, 22-27] and 2 cross-sectional studies.[28, 29] Twelve studies determined the cyclic variation of PAI-I, t-PA and/ or uPA,[11-14, 18, 22-26, 28] five studies assessed the cyclic variation of d-dimer[7, 12, 14, 26, 29] and one study determined α2-antiplasmin levels during the menstrual cycle.[27]

All studies included healthy volunteers. Study sizes ranged from 9 to 41 women. The number of samples in the longitudinal studies varied from 2 to 9 during menstrual cycle and the days of sampling

were different between the studies. In 11 out of 15 studies the menstrual cycle was established as ovulatory by measuring hormones.[7, 11-13, 20, 22-25, 28, 29]

Two out of 11 studies found cyclic variation of PAI-I.[12, 22] Giardina et al.[12] found the lowest levels during the luteal phase (day 28), whereas Chung et al.[22] found the lowest levels during the follicular phase (days not specified). One out of 10 studies found cyclic variation of t-PA.[25] Ricci et al.[25] found the lowest levels of t-PA during the luteal phase (day 21). Two out of 3 studies found a cyclic variation of uPA.[22, 28] Chung et al.[22] found the lowest levels during the ovulatory phase (days not specified) and Dorr et al.[28] during the post-ovulatory phase (day 19-22). Two out of five studies found a cyclic variation of d-dimer during the menstrual cycle.[12, 14] Koh et al.[14] found the lowest levels late follicular (day 10-14) whereas Giardina et al.[12] found the lowest d-dimer levels in luteal phase (day 28). Additionally, we found one study by Jespersen et al.,[27] which described a longitudinal study about α2-antiplasmin during the menstrual cycle. They found significantly lower levels during the late follicular phase (day 12-16).

Figure 3: Fibrinogen (g/l) levels during follicular and luteal phase



* = significant variation of fibrinogen levels

Fibrinogen

We identified 20 studies that tested fibrinogen during the menstrual cycle. See for detailed information about the studies table 5._We found 17 longitudinal studies,[7, 8, 10-12, 14, 18, 20, 23-25, 30-35] one study with a cross-sectional and a longitudinal part,[17], (17) one randomized controlled trial in which

the haemostatic variables were measured longitudinally during the menstrual cycle in the placebo group[26] and one cross-sectional study.[9] The study sample sizes varied from 10 to 350 women. In the longitudinal studies, women were sampled two to sixteen times. All studies included healthy volunteers, except the study by Kadir et al.[17] which included also 123 patients with menorrhagia. In total, 12 studies determined the menstrual cycle by measuring hormones.[7-12, 20, 23-25, 34, 35]

Six out of 20 studies reported the lowest levels of fibrinogen during the follicular or mid-cycle phase.[7, 14, 17, 24, 32, 34] Two studies reported the lowest levels during luteal phase[25, 35] and all other studies reported no cyclic variation.[8-12, 18, 20, 23, 26, 30, 31, 33] Figure 3 shows variation of fibrinogen levels during follicular and luteal phase.

Table 5: Fibrinogen during the menstrual cycle.

Study	Number of patients	Hormone analysis Y/N	Levels lowest in (cd or phase)	Measurement (cd or phase)				
				1	2	3	4	5
Roell et al, 2007	52	Y	NA	F	L			
Koh et al, 2005	30	N	10-14	1-3	5-9	10-14	21-26	
Giardina et al, 2005	20	Y	NA	F	L			
Ricci et al, 2004	41	Y	21	1	7	14	21	
Dapper et al, 2002	350	N	F	M	F	O	L	
Repina et al, 2002	10	Y	NA	F	L			
Feuring et al, 2002	18	Y	8-11	8-11	18-21			
Kadir et al, 1999	142 †	N	11	1	11	26		
He et al, 1999	23	N	NA	5-7	24-26			
Blomback et al, 1997	15	Y	NA	5-7	20-25			
Spona et al, 1997	20	N	NA	7	14	21		
Larsen et al, 1996	10	Y	O	Twice weekly samples during 9 weeks				
Blomback et al, 1992	15	Y	NA	unknown				
Lebech et al, 1989	37	Y	10-12	6-8	10-12	24-26		
Siegbahn et al, 1989	13	Y	NA	early F	late F	early L	late L	M
Solerte et al, 1988	15	Y	25	7	14	21	25	27
Jespersen et al, 1986	15	Y	NA	1-3	5-7	12-16	19-23	27-30
Gaur et al, 1982	18	N	NA	1-7	8-14	15-21	22-28	
Buchan et al, 1980	12	N	NA	1-7	12-16	18-21	26-28	
Cederblad et al, 1977	18	N	NA	1-2	5-9	12-16	19-23	

Y= yes; N= no; NA= not applicable (no cyclic variation) † = 123 of them were menorrhagia patients; F= follicular phase; O= ovulation; L= luteal phase; M= menstruation

Discussion

In this study, we performed a systematic review of the variation of different haemostatic variables during the menstrual cycle. These studies yielded conflicting results. The majority of the studies observed no cyclic variation, but if there was a variation, the lowest levels were measured almost always during menstruation and early follicular phase. For increasing the sensitivity of haemostatic testing, we suggest, based on these data that the optimal timing for haemostatic testing during the menstrual cycle seems to be during menstruation or early follicular phase for vWF, factor VIII and platelet function tests. For fibrinolytic parameters we found in most of the studies no cyclic variation during menstrual cycle.

To our knowledge, this is the first reported systematic review concerning the variation of different haemostatic variables during menstrual cycle. We focused on several haemostatic variables, including platelet function, von Willebrand factor, factor VIII, factor IX, factor XI, factor XIII, fibrinolytic factors (α2-antiplasmin, PAI, t-PA, uPA, d-dimer) and fibrinogen which may cause a bleeding tendency when women have lower levels during menstruation.

Both studies that performed PFA testing during the menstrual cycle, found the lowest platelet functioning during the follicular phase and an increase during the luteal phase.[7, 9] This is probably due to an increase of vWF-levels in response to progesterone actions.[9]

Five out of eleven studies[9, 11, 16, 17, 19] reported the lowest vWF levels during menstruation or early follicular phase (cd 1-7). For FVIII we found the same results, two out of nine studies reported the lowest levels during menstruation and early follicular phase[16, 19] and the other studies reported no cyclic variation. In case of borderline vWF-levels measured randomly or independently of the menstrual cycle in a woman with menorrhagia, we suggest to repeat testing during menstruation, preferable during the first 3 days. In addition, this could give therapeutic options for these patients, because it is during those initial 3 days that the patient would be a candidate for intranasal desmopressin for raising low vWF levels.[36] Lower levels of von Willebrand factor and FVIII levels during this phase are possibly due to the fact that most female hormones are at their baseline in the early follicular phase.

We found 15 studies about fibrinolytic parameters during menstrual cycle. Menstrual bleeding is associated with an increase in local fibrinolysis due to elevated levels of endometrium derived plasmin and plasminogen activators.[37] However, most of the studies (10 out of 15) reported no cyclic variation of fibrinolytic parameters in plasma. Two studies found cyclic variation of d-dimer, two studies of PAI-I, two studies of uPA and one study of t-PA. We found one study about α2-antiplasmin, in which the lowest levels were reported during late follicular phase.[27] Therefore, we assume that most fibrinolytic parameters have no significant cyclic variation during the menstrual cycle.

Our data about fibrinogen levels during the menstrual cycle showed conflicting results. Six out of the 20 studies reported the lowest fibrinogen levels during the follicular or mid-cycle phase. Two studies reported the lowest levels during the luteal phase and all other studies reported no cyclic variation. Fibrinogen is an acute phase reactant, which can increase 2-20 fold during inflammation

and stress with a peak elevation that occurs after 3-5 days of onset. The strong association between fibrinogen and the acute phase reaction could be an explanation for these conflicting results.

A limitation of the included studies is that the days of sampling in the menstrual cycle differed between most of the studies. All studies measured the haemostatic variables during the follicular and luteal phase, but some studies measured it on more different moments, varying from two to sixteen times per cycle. Accordingly, because of obvious heterogeneity we could not pool these data. Moreover, most of the studies had a longitudinal design, but we included also 4 studies[9, 17, 28, 29] with a cross-sectional design and 1 retrospective study with a cross-sectional design.[16] In addition, the haemostatic variables were not always correlated to estrogen and progesterone levels. Consequently, the menstrual cycle was not always confirmed to be ovulatory.

Secondly, another limitation of our study is that most of the described studies on the variation of haemostatic variables studied small patient numbers. Thus, real differences between the different phases of the menstrual cycle may be missed, because of the wide inter- and intra-individual biological variation in haemostatic factor levels.[10] Moreover, a small number of patients could lead to a high coefficient of variation (CV) of the used laboratory tests. If the difference in the level of the haemostatic variable was lower than the CV, it is possible that the reported significant difference is not a real difference, but a difference what is caused by the small number of patients. For example we calculated the CV of laboratory tests which were used for the vWF measurement in the different studies. VWF-levels were mostly about 10% lower during menstrual/ follicular phase, while the CV was in almost all studies about 30%. On the other hand, five out of eleven studies observed the same results for vWF, which gives us support to perform haemostatic evaluation during menstrual and follicular phase in clinical practice.

Thirdly, an important part of the wide variation in results is due to the use of a variety of laboratory assays for determining the different haemostatic parameters in the included studies.

In only one study, by Kadir et al.[17] also women with menorrhagia were included. They found no cyclic variation for VWF, FVIII, FXI and fibrinogen during menstrual cycle. In the future we need studies for evaluating haemostatic variables during normal menstrual cycle and in women with menorrhagia, because perhaps women with menorrhagia show more or less variation. This could give us more insight in the pathogenesis of menorrhagia.

In conclusion, this systematic review of the literature shows that the optimal timing of haemostatic testing seems to be the menstrual and early follicular phase of the menstrual cycle for most haemostatic variables. Of note, most of the studies found no cyclic variation, but if they showed a variation, the lowest levels were measured during these phases. Physicians suspecting deficiencies in haemostatic factors should therefore test during these phases, thereby increasing the sensitivity of the test.

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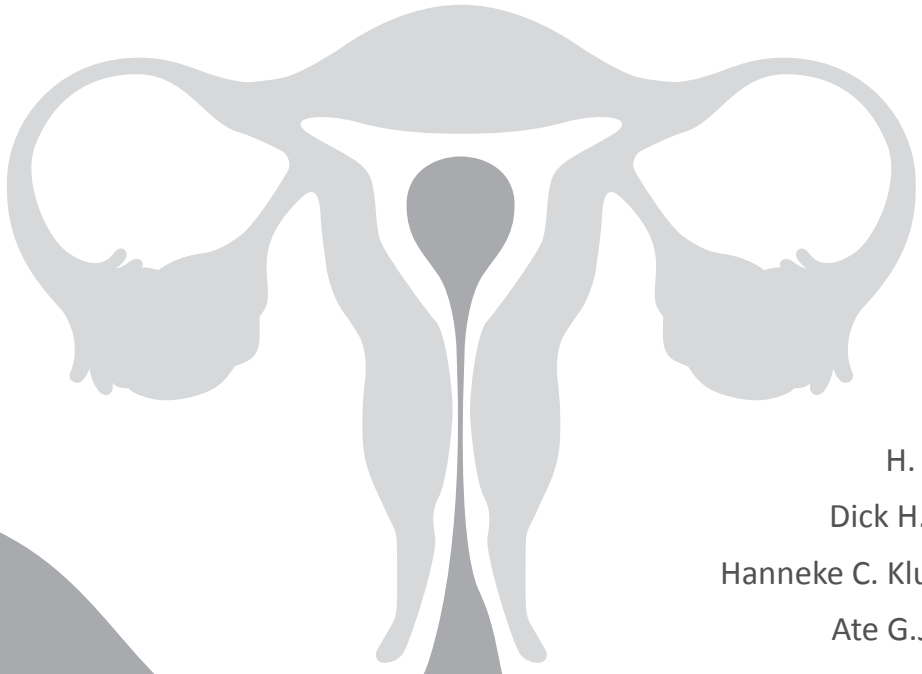
Chapter 2

Routine evaluation and treatment of unexplained menorrhagia: do we consider haemostatic disorders?



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Abstract

Objective: unexplained menorrhagia can be caused by underlying bleeding disorders. Aim of this study was to investigate the current work-up of menorrhagia in routine gynaecological practice, with a special interest in haemostatic evaluation. Secondly, we investigated the outcome of individualised treatment in our centre.

Study design: retrospective medical chart review of 112 consecutive patients referred for menorrhagia to a general gynaecology clinic of a university teaching hospital in the Netherlands between January 2006 and January 2007. In April 2008 we performed a structured telephone interview evaluating the effectiveness of their therapy.

Results: we included 112 patients, median age was 42 years. Twenty-nine percent were anaemic (hemoglobin<12.0 g/dL). Seventy-one (63%) patients had unexplained menorrhagia. Only two patients had haemostatic evaluation, both had no von Willebrand`s disease. Forty percent (29/71) needed 2 or more different therapies, 17% (12/71) needed 3 different therapies and 2 patients needed a total of 7 different therapies. Eight patients underwent a hysterectomy, six of them after endometrial ablation. Most patients (80%) were medically or surgically successfully treated and were satisfied with their therapy during follow-up. Eleven patients declined therapy and accepted their heavy periods.

Conclusion: haemostatic evaluation in women with unexplained menorrhagia in gynaecological practice is uncommon in our centre. Although most of the patients were satisfied with their treatment, a significant number had required hysterectomy and another important proportion had to accept their menorrhagia. We hypothesize that the identification of haemostatic disorders might improve care for these women.

Introduction

Menorrhagia is a common problem among women in the reproductive age. At least 5-10% of women in reproductive age will seek medical attention for menorrhagia.[1] The World Health Organization estimates that 18 million women worldwide are affected.[2,3] Menorrhagia is a common cause of iron deficiency anaemia[4, 5] and may affect a woman's quality of life, her study or work and family and social interactions.[6] Menorrhagia can be caused by a wide range of disorders.[7,8] For women with menorrhagia without gynaecological abnormalities a haemostatic evaluation for underlying bleeding disorders,[2,9] including von Willebrand`s disease, platelet dysfunction and coagulation factor deficiencies has been advised,[10] although the impact of testing on patients treatment outcome parameters has not been studied.[9, 11] In the absence of carcinoma or other significant pelvic pathology, medical treatment is generally the first line of therapy for women with menorrhagia.[12] The aim of this study was to investigate the work-up of menorrhagia in the University Medical Centre of Groningen, with a special interest in haemostatic evaluation. Secondly, we assessed the outcome of routine treatment in patients with unexplained menorrhagia.

Materials and Methods

We retrospectively identified all consecutive patients with menorrhagia referred to the general gynaecology clinic of the University Medical Centre of Groningen between January 2006 and January 2007, by searching computerized hospital files for the Dutch diagnosis-and-treatment code (DBC code), G11, (menstrual cycle disorders, including post menopausal bleeding). For all thus identified patients, medical records were reviewed. National legislation and the ethical committee of our institution approve this type of retrospective study without the need for review of the protocol. Menorrhagia was defined as heavy regular menstrual bleeding.[13] Patients with postmenopausal, irregular, postcoital and intermenstrual bleeding were excluded. Medical records contained detailed information on gynaecological history elicited by using a standardized form and reviewing past medical history. All patients had a gynaecological examination and a pelvic sonography. Saline infusion sonography, hysteroscopy, haemostatic evaluation and other tests were performed when clinically indicated. Patients with intracavitary polyps, submucosal myoma or intramural myoma more than 2 cm in diameter were classified as having a gynaecological abnormality or explained menorrhagia. We collected data including age at presentation, onset of menorrhagia, menorrhagia since menarche, duration of menstrual period, previous history of anaemia, associated medical problems such as hypothyroidism, use of medications (including aspirin and vitamin K antagonists), abnormalities during gynaecological examination, anaemia during presentation, laboratory data on bleeding disorders, initiated treatments, effectiveness and satisfaction with treatment. Specifically, we recorded whether the gynaecologist had documented a bleeding history, included post-partum haemorrhage, nose bleedings and bleeding after dental or other procedures. In routine care, an individualized treatment plan was made for each patient with unexplained menorrhagia, mostly starting with medical therapy.

Choices depended on the preferences of the patient, the effectiveness of any previous therapy, fertility plans and contra-indications for a given drug. Options included hormonal treatment and non-hormonal treatment. Hormonal treatments were combined oral contraceptives (COCs), cyclical and continuous progestogens and a levonorgestrel-releasing intrauterine device (Mirena-IUD). Non-hormonal treatments were non-steroidal anti-inflammatory drugs (NSAIDs) and tranexamic acid during menstruation. Surgical management (endometrial ablation or hysterectomy) was considered if previous treatments had failed or patients did not tolerate or choose medical treatments.

In April 2008 we contacted all included patients with unexplained menorrhagia by telephone using a standardized questionnaire about symptoms and effectiveness (effective – partially effective – not effective) of both the initial and – if applicable – the most recently started therapy. We classified a treatment as effective if a patient was satisfied, considered her periods as normal and wanted to continue the therapy.[14] We analyzed the data with Statistical Package for the Social Sciences (SPSS) version 16.0.

Results

In the computer search, we identified 769 patients between January 2006 and January 2007 with the DBC code G11. We excluded 657 patients for postmenopausal, irregular, postcoital or intermenstrual bleeding. One hundred and twelve patients were identified with the diagnosis menorrhagia. All their medical records were reviewed with a standardized data form. Patient characteristics are shown in Table 1. Median age was 42 years (range 13-54 years) and the median age of the onset of menorrhagia was 39 years. Seven patients (6%) had menorrhagia since the menarche. The duration of the menstrual period varied, the median was 7 days. A previous history of anaemia was reported by 36 patients (32%). At the time of testing 29% (32/112) of the patients were anaemic (hemoglobin <12.0 g/dL). In seventy-one patients (63%), no gynaecological abnormality was diagnosed (unexplained menorrhagia), 16 patients (14%) had an intracavitary abnormality (endometrial polyp in 12, submucosal uterine myoma in 4) and 25 patients (22%) had intramural myoma. Personal history of bleeding after surgery, tooth extraction, delivery or miscarriage was reported in one patient, who had a postpartum haemorrhage. Five patients reported a family history of menorrhagia. A laboratory evaluation for underlying bleeding disorders was performed in two patients, one of whom had menorrhagia since menarche and the other had a postpartum haemorrhage. Von Willebrand`s disease was excluded in both. Six patients had a history of hypothyroidism, but all used thyroid replacement therapy. Four patients had menorrhagia while on antithrombotic therapy. Three patients used a vitamin K antagonist, two of them had also a gynaecological abnormality. One patient used dipyridamol, a platelet inhibitor in combination with a vitamin K antagonist.

Table 1: Characteristics of study population

	All patients (n = 112)	No gynaecological abnormality (n=71)	Gynaecological abnormality (n=41)
Age at enrolment (years)	42 (13-54)	41(13-51)	43 (26-54)
Menstrual period (days)	7 (4-17)	7 (4-17)	7 (4-16)
Menorrhagia since menarche	7	5	2
Anaemia	32	14	18
Antithrombotic therapy	4	2	2
Hypothyroidism (suppleted)	6	6	0
Haemostatic testing	2	2	0
Postmenopausal during follow-up	6	1	5

Continuous variables are denoted as median (range), categorical variables as number.

Initial therapy - unexplained menorrhagia

In each case of unexplained menorrhagia (n=71) treatment was tailored to the individual patient. Of the patients with unexplained menorrhagia, fifty were initially treated with hormonal treatment. Seventeen of them were treated with cyclical or continuous progestogens, 20 patients with a Mirena-IUD, twelve patients with COCs and one patient with danazol. Fifteen patients were initially treated with non-hormonal medicines; five patients with tranexamic acid, eight patients with NSAIDs and two patients with a combination of NSAIDs and tranexamic acid. One patient underwent an endometrial ablation and five patients declined therapy.

Initial therapy - gynaecological abnormalities

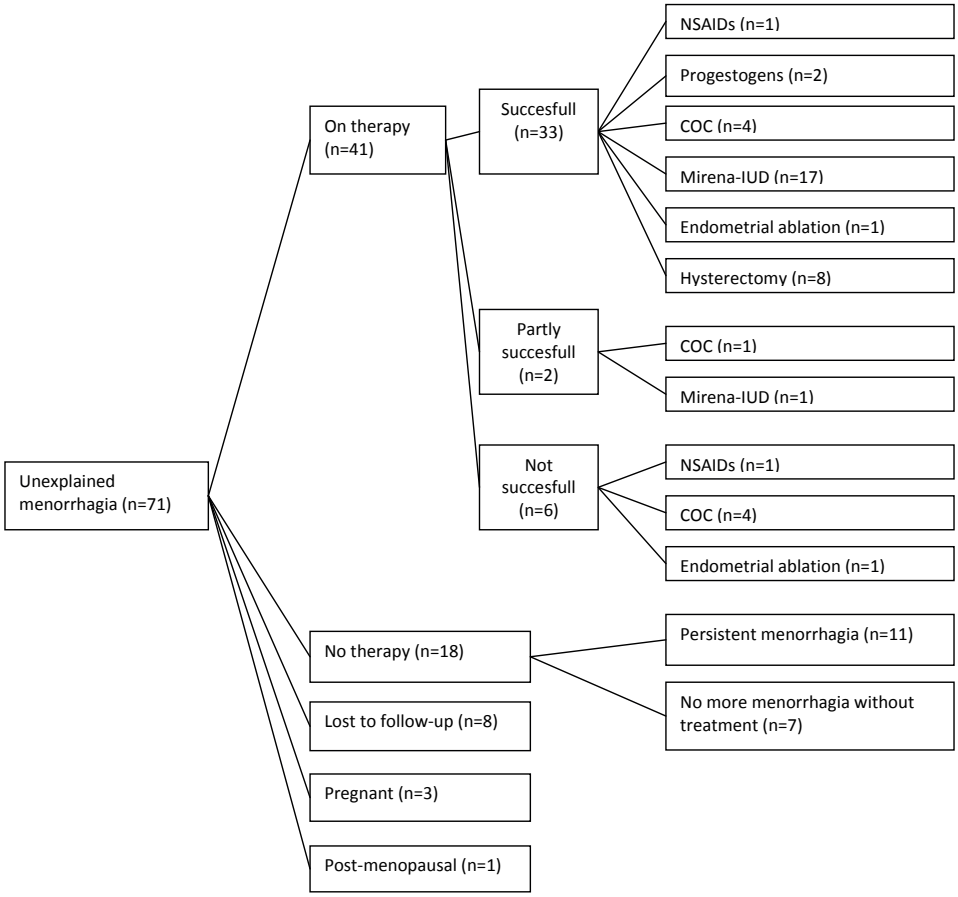
Of the patients with gynaecological abnormalities (n=41), 19 patients underwent a therapeutic hysteroscopy (polyp resection in 12, myoma resection in 7), one patient an uterine artery embolisation, one patient a myomectomy and one patient a hysterectomy. Twelve patients were initially treated with hormonal treatment, six of them with a Mirena-IUD, four with progestogens, one with COCs and one with danazol. Seven patients were initially treated with non-hormonal treatment, four of them with NSAIDs and three with tranexamic acid.

Treatment and follow-up during telephone interview in patients with unexplained menorrhagia

Outcome in all patients with unexplained menorrhagia (n=71) was assessed by a structured telephone interview. Eight patients of them were lost to follow-up. The follow-up period from the first clinic visit to the telephone interview ranged from fourteen to twenty-six months. Figure 1 shows the distribution of the therapies of patients with unexplained menorrhagia during follow-up. Of the patients with unexplained menorrhagia who were still on treatment 80% (33/41) were satisfied and

20% (8/41) were partly or not satisfied at follow-up. Forty percent (29/71) needed 2 or more different therapies, 17% (12/71) needed 3 different therapies and 2 patients needed a total of 7 different therapies. Further, 15% (11/71) of the patients declined therapy and accepted their heavy periods, 10% (7/71) had normal periods without current therapy and 17% (12/71) were pregnant, post-menopausal or lost to follow-up. Of the 14 patients with anaemia before therapy, nine patients had improvement in anaemia with therapy, one patient had still anaemia while she accepted her heavy periods, hemoglobin level with therapy was missing in one patient and three patients were lost to follow-up. A total of nine patients with unexplained menorrhagia underwent an endometrial ablation, in six of them followed by hysterectomy. In total, 8 (11%) patients underwent a hysterectomy. No patient needed a blood transfusion or developed excessive bleeding with surgery or during therapy.

Figure 1: Treatment of patients with unexplained menorrhagia at follow-up



Comment

Our study shows that routine haemostatic testing of patients with unexplained menorrhagia (no gynaecological abnormalities) referred to a university teaching hospital is uncommon. Only two patients of this cohort have had haemostatic evaluation for von Willebrand`s disease, which was negative in both. Despite the absence of haemostatic evaluation, most patients were satisfied with their treatment.

In our cohort two patients were tested for only von Willebrand`s disease and not for other bleeding disorders such as platelet dysfunction. In addition, two patients with menorrhagia without gynaecological abnormalities had menorrhagia while on antithrombotic therapy, one due to a platelet inhibitor in combination with a vitamin K antagonist and one due to a vitamin K antagonist. In one of the two patients who had haemostatic testing a postpartum haemorrhage was recorded as a bleeding symptom and the other patient had menorrhagia since menarche. It is possible that gynaecologists do not routinely ask for other bleeding symptoms, so are not prompted to think of underlying bleeding disorders in women with menorrhagia, which could also explain the low frequency of haemostatic testing in our cohort. Another reason that testing for haemostatic disorders is still not widespread in the gynaecological setting is that first line therapy (i.e. combined oral contraceptives) is not influenced.[15] On the other hand specific therapies are available for von Willebrand`s disease (desmopressin) and fibrinolytic disorders (tranexamic acid).[16] No studies have reported on the frequency of testing for haemostatic disorders in adult women with unexplained menorrhagia. One study is available in adolescents, which also reported that the majority (85%) of the adolescents were not screened for bleeding disorders.[17] Sixty-three percent of the patients in our cohort had unexplained menorrhagia, which is comparable with several studies.[7,8,13] However a proportion of these patients might have an underlying bleeding disorder. The prevalence of von Willebrand`s disease has been reported in approximately 1:1500 of the general population.[18,19] In women with menorrhagia the prevalence of von Willebrand`s disease varies ranging from 5 to 24%,[9, 11, 20] with an overall prevalence of 13% (95% confidence interval, 11-16%) based on a systematic review of 11 studies comprising 988 women with menorrhagia.[20] Philipp et al. demonstrated bleeding disorders in 47% of 115 women with menorrhagia. This study also suggested that the prevalence of platelet dysfunction may be even higher than von Willebrand`s disease in women with menorrhagia.[21,22] They found no differences in prevalence between adolescents, reproductive-aged women and perimenopausal women.[22, 23]

We have chosen to take satisfaction as measurement of the effectiveness of the therapy. This is in line with a Cochrane systematic review which concludes that the definition of treatment success in patients with menorrhagia is perhaps best based on woman's satisfaction level and willingness to continue a particular therapy.[14] Outcomes measured by methods like the alkaline haematin technique[24] and the pictorial blood loss assessment chart (PBAC score)[25] are less patient-important, although they are valuable from clinician's point of view. In our centre the therapy was individualized per patient and depending on the preferences of the patient, the satisfaction and side-effects of any previous

therapy, but also on fertility plans and contra-indications for a given drug. So a wide range of routine medical treatments were used to reduce menstrual bleeding. Surgical management (endometrial ablation or hysterectomy) was considered if patients did not tolerate or choose medical treatments or where such treatments had failed. Of the patients who were treated for menorrhagia, most of them (80%) were satisfied. There are no recent studies of present care in general for women presenting with chronic menorrhagia. In general, progestogens are the most prescribed therapy for women with menorrhagia. A Cochrane systematic review showed that cyclical progestogens are less effective than other medical therapies such as danazol, tranexamic acid, NSAIDs and the Mirena-IUD.[8] Progestogens for 21 days of the cycle resulted in a significant reduction in menstrual blood loss, although women found the treatment less acceptable than Mirena-IUD.[8] Probably cyclical progestogens are a good option for short-term treatment.[8] In our cohort most of the patients with a Mirena-IUD were satisfied, but the number of patients per treatment was too small to analyze. Although most of our patients were satisfied with their treatment, this required a hysterectomy in 8 patients. Also $\geq$ 2 or $\geq$ 3 different therapies were needed in 29/ 71 patients and 12/ 71 patients respectively.

In conclusion, haemostatic evaluation in patients with unexplained menorrhagia is uncommon in our centre. Managing unexplained menorrhagia without testing for haemostatic disorders does not compromise patient satisfaction. However, treatment is not optimally efficient, requiring often several lines of therapy, and more cases of hysterectomy should be avoided. We hypothesize that systematic haemostatic testing might improve patient care by identifying disorders that respond to specific therapies. Presently, we are undertaking a prospective study to test this hypothesis.

Acknowledgement

We regret to report that prof. dr. J. van der Meer passed away during the final phase of the manuscript.

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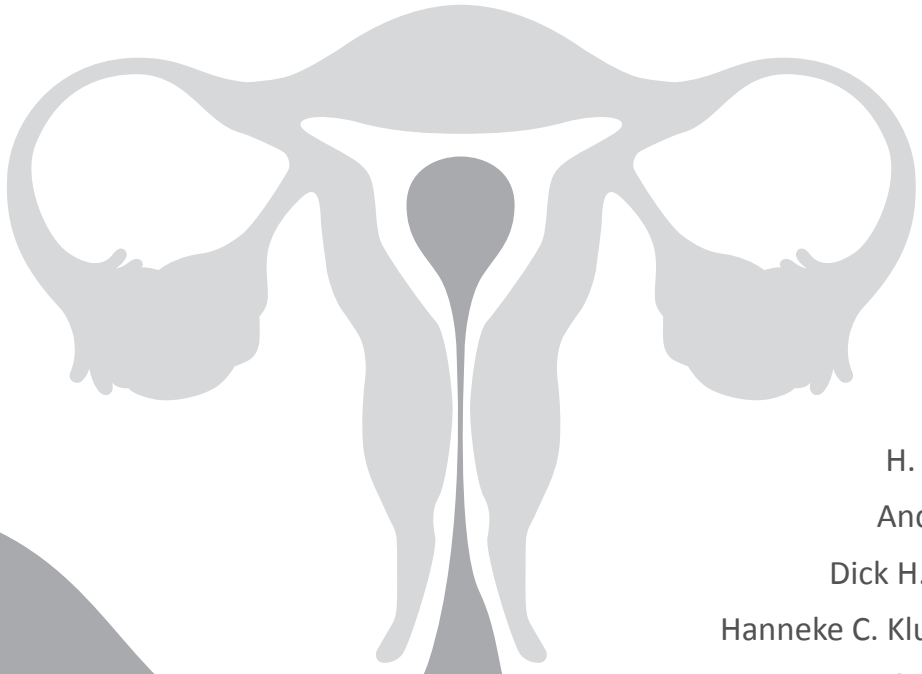
Chapter 3

The prevalence of underlying bleeding disorders in patients with menorrhagia with and without gynaecological abnormalities



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Submitted

Abstract

Objective: to assess the prevalence of underlying bleeding disorders in women with menorrhagia with and without gynaecological abnormalities.

Design: single-centre prospective cohort study

Setting: a tertiary referral teaching hospital

Population: hundred-and-two consecutive patients referred for menorrhagia with a PBAC-score >100 were included. Controls are 28 healthy volunteers without menorrhagia.

Methods: patients and controls had haemostatic testing in the 1st week after menstruation. Patients underwent gynaecologic evaluation.

Main measure outcomes: prevalence of von Willebrand disease, FXI deficiency, platelet defects and other coagulation factor deficiencies.

Results: twenty-six percent of the patients had gynaecological abnormalities, sufficient to explain menorrhagia. An underlying bleeding disorder was found in 29% versus 11% (p=0.04) of patients versus controls, and in 31% vs 27% of the women with unexplained versus explained menorrhagia (p=0.75). We diagnosed 6 cases of VWD, 4 cases of FXI deficiency and one FVII deficiency. The only abnormalities found in controls were platelet aggregation defects (11% versus 23% in patients). Patients had a significantly longer aPTT compared to controls (26.5 vs 25.0 sec; p=0.001) caused by lower median levels of FXI (100 versus 124 IU/dL; p<0.001).

Conclusion: bleeding disorders play an equally important role in the aetiology of menorrhagia with and without gynaecological abnormalities. A novel finding is the occurrence of low, but not deficient levels of factor XI.

Introduction

Menorrhagia is a common problem. At least 5-10% of women in reproductive age seek medical attention for menorrhagia.[1] The World Health Organization estimates that 18 million women worldwide are affected.[2] Menorrhagia is a common cause of iron deficiency anemia[3] and can affects a woman's quality of life, her study or work and family and social interactions.[4]

Menorrhagia can be associated with a wide range of haemostatic disorders.[5,6] Von Willebrand`s disease (VWD) has been recognized as an important aetiologic and/or contributory factor.[7-9] About 13-20% of the patients with menorrhagia without gynaecological abnormalities have VWD as underlying bleeding disorder.[10] The prevalence in menorrhagia of underlying bleeding disorders other than VWD, for example platelet function defects, is yet to be established. Although a cyclic variation of haemostatic factor variables has been found[11] with nadirs occurring during the menstrual and/ or follicular phase, most previously reported studies have measured variables randomly throughout the menstrual cycle. This may result in over- or underestimation of the prevalence of a bleeding disorder.[10] Previous studies have focussed on women without gynaecological abnormalities. Whether women with menorrhagia and gynaecological abnormalities as uterine polyps and fibroids also have underlying bleeding disorders has not been assessed in earlier studies.[10]

We studied the prevalence of underlying bleeding disorders, including VWD, other coagulation disorders and platelet defects, in patients with menorrhagia with and without gynaecological abnormalities with testing in the 1st week after menstruation in routine Dutch gynaecological practice.

Methods

Patients

This is a single-centre prospective cohort study, including consecutive patients referred to the gynaecology clinic at the University Medical Centre of Groningen between March 2007 and December 2010, with a history of heavy, regular (every 23–39 days) menstrual periods. Exclusion criteria were: PBAC-score <100, known bleeding disorders, use of any intrauterine device in the past 2 months, and treatment with anticoagulants, antifibrinolytics, non-steroidal anti-inflammatory agents, combined oral contraceptives, or progestagens. Referred patients who were potentially eligible received a structured questionnaire by mail to obtain information about baseline characteristics; medical, obstetrical and gynaecological history and previous treatment for menorrhagia. After reviewing the completed questionnaire, we excluded women with intermenstrual, irregular and postcoital bleeding. Thereafter they had a gynaecological examination and pelvic ultrasonography in the first week after menstruation. One interviewer (HMK) took the menstrual history, recorded the number of other bleeding symptoms such as easy bruising, nose, gum, postoperative, and postpartum bleeding, and bleeding after tooth extraction, and asked about a family history of bleeding disorders. Women with submucous uterine fibroids more than 2 cm in diameter and/or uterine polyps were classified as

menorrhagia with gynaecological abnormalities, or explained menorrhagia.

The study was approved by the Institutional Review Boards of the University Medical Centre of Groningen. Informed consent was obtained from all patients and controls.

Controls

We also recruited 28 healthy volunteers in our hospital; women without menorrhagia (defined as subjectively normal menstruation) and without the use of hormonal treatment or intra-uterine device, for comparison of haemostatic test results in the first week after menstruation during follicular phase. Exclusion criteria were: known bleeding disorders, and treatment with anticoagulants or non-steroidal anti-inflammatory agents. Controls completed the same questionnaire as the women with menorrhagia, but had no gynaecological examination. We did not exclude controls on the basis of their PBAC score.

Pictorial bleeding chart assessment of menorrhagia

Patients were informed about the pictorial bleeding assessment chart (PBAC)[12,13] by an information letter with standard instructions before the first hospital visit and completed it in the menses before the first hospital visit. Women were instructed to use maxi tampons and pads. The chart consisted of a series of diagrams representing lightly, moderately and heavily soiled pads and tampons. The patients recorded each discarded item during an entire cycle. Scoring of pads and tampons was done by the interviewer (HMK) as previously described [12, 13] with tampons scored 1, 5 and 10 and pads scored 1, 5, 20 for lightly, moderately and heavily soiled, respectively. Menorrhagia was defined as a pictorial bleeding assessment chart (PBAC) score of 100 or more based on the scoring system of Higham et al.[12] The healthy volunteers also completed the pictorial chart, in the first menses after blood samples were taken.

Laboratory measurements

A venous citrated blood sample was taken from all patients and controls in the first week after menstruation. In patients the blood samples were taken before the gynaecological examination. Blood samples were also obtained for ABO blood group typing, complete blood cell counts, ferritin and liver, kidney and thyroid function measurements.

We measured activated partial thromboplastin time (aPTT) with reagents (Dade® Actin ®FS Reagent) obtained from Siemens (Marburg, Germany), prothrombin time (PT) with reagents (Dade® Innovin® Reagent) obtained from Siemens, fibrinogen with Dade® Thrombin Reagent from Siemens. The one-stage factor VIII, IX, XI and XII assays were performed with APTT reagents (Dade® Actin ®FS Reagent) and factor deficient plasmas, obtained from Siemens. α_2-antiplasmin (PI) measurements were performed with Berichrom® and PI reagents were obtained from Siemens. All assays were measured on a CA-7000® system (Sysmex Corporation, Siemens). Von-Willebrand-factor antigen (vWF:Ag) was measured

by ELISA using polyclonal antiserum from DakoCytomation (Glostrup, Denmark), von-Willebrand-factor ristocetin cofactor activity (vWF:Rco) was measured with Von Willebrand Reagent (lyophilized stabilized platelets and ristocetin) from Siemens in an optical aggregometer from Chrono-Log Corp (Haverton, PA, USA).

Induced platelet aggregation measured by light transmission aggregometry (LTA; Chrono-Log Corp) was performed at 37.8°C in platelet-rich plasma with 5 different agonists; ADP 3.3 micromol/mL (Sigma-Aldrich B.V., Zwijndrecht, the Netherlands), ristocetin 1.2 mg/mL (Sigma-Aldrich), arachidonic acid 1.5 mmol/L (Chron-Log Corp), epinephrine 1 microgram/mL (Pharmachemie B.V., Haarlem, the Netherlands), and collagen 1 microgram/mL (Chro-Log Corp). In a subgroup (n=48), we also performed LTA with lower ADP concentrations of 1.0, 1.5, and 2.0 micromol/mL. Aggregation tracings were performed for 10 minutes and maximal percent aggregation was recorded. Reference values (mean +/- 2SD) were estimated in the control group on log transformed data and a decreased aggregation was defined as a maximal percent aggregation below the mean-2SD. Lower limit of normal reference values (mean-2SD) were for ADP (3.3 micromol/mL) 64%; ristocetin 72%; epinephrine 70%; collagen 70% and arachidonic acid 69%.

For the other haemostatic parameters the normal ranges in our laboratory were: PT 9-12 sec; aPTT 23-33 sec; fibrinogen 1.7-4.0 g/l; FVIII:C 50–150 IU/dL; FIX 50-150 IU/dL; FXI 70–130 IU/dL; FXII 65-150 IU/dL; vWF:Ag 50–150 IU/dL; vWF:Rco 50–150 IU/dL and PI 75-125 IU/dL.

Values below the lower limit of normal reference range were confirmed by a second, independent sample. A diagnosis of von Willebrand's disease was made if vWF:Ag or vWF:Rco was less than 50 IU/dL in two measurements.

Statistical analysis

Continuous variables, expressed as medians (ranges) were used for age, age of menarche, duration of period and number of days of heavy menstrual bleeding. To analyse differences in levels of hemoglobin, platelet count, MCV and ferritin in women with menorrhagia and controls Mann Whitney U tests were used. The chi-square test was performed to analyse differences between the prevalence of bleeding disorders of women with unexplained menorrhagia and explained menorrhagia, respectively. Because the levels of all haemostatic variables and percentage of maximal aggregation were non-normally distributed, we log transformed them. Differences in log transformed means between women with menorrhagia and controls were evaluated by t-tests. ANOVA test was used to analyse differences in the number of decreased maximal aggregation in patients and controls. A P-value of less than or equal to 0.05 was considered statistically significant.

Results

Baseline characteristics

We included 102 consecutive patients with menorrhagia and 28 healthy volunteers. We excluded 139 patients with PBAC <100, irregular periods, postcoital bleeding, usage of contraceptives or anticoagulants. Median age was 42.5 yrs (range 17-55) in patients and 40.0 yrs (range 25-55) in controls (p=0.06). Ninety-five percent of the patients were Caucasian. Median PBAC score was 271 in patients vs 126 in controls (p<0.001). Anaemia (Hb < 7.5 mmol/L) was more prevalent in patients compared to controls (46 vs 9%, p<0.000). Patients had a lower median Hb (7.7 vs 8.4 mmol/L; p<.001), a lower median MCV (84 vs 89 10^{-15}L, p<0.001), lower median ferritin levels (12 vs 34 ng/mL; p<0.001) and a higher median platelet count (289 vs 251 10^{9}/L, p<0.001). Seventy-four percent had unexplained and 26% of patients had explained menorrhagia (with a gynaecological abnormality), respectively. See for detailed information table 1.

Table 1: Baseline characteristics in menorrhagia patients and controls

	Patients (n=102)	Controls (n=28)	p-value
Median age, yrs (range)	43 (17-55)	40 (25-55)	0.06
Median age of menarche, yrs (range)	13 (9-17)	13 (11-14)	0.68
Median age first symptom menorrhagia	33 (9-54)		
Median duration period, days (range)	7 (3-15)	5 (3-14)	<0.001
Median length of cycle, days (range)	28 (22-37)	28 (23-34)	0.48
Median duration menorrhagia, months (range)	79 (3-436)		
Race, n (%)			
White	97 (95)	28 (100)	0.58
Black	1 (1)	0	
Asian	4 (4)	0	
PBAC-score, median (range)	271 (106-823)	126 (41-290)	<0.001
Hemoglobin < 7.5 mmol/L, n (%)	47 (46)	2 (9)	<0.001
Ferritin < 15 ng/mL, n (%)	62 (61)	2 (9)	<0.001
Gynaecological abnormalities, n (%)	26 (26)		

Bleeding disorders

Overall, we found in 29% of the patients vs 11% of the controls an underlying bleeding disorder (p=0.04). The only bleeding disorder identified in controls was decreased maximal platelet aggregation with one or more agonists. Thirty-one percent of the patients without vs 27% with gynaecological abnormalities had an underlying bleeding disorder (p=0.48). VWD was diagnosed in 7 vs 4%, respectively (p=0.58). Low FXI levels (<70%) were found in 4% of the women with unexplained and in

4% of the women with explained menorrhagia. One patient with unexplained menorrhagia exhibited a FVII deficiency (FVII = 49 IU/dL). Decreased maximal platelet aggregations with one or more agonists were found in 21% women with unexplained vs 27% with explained menorrhagia (p=0.36). See also table 2.

Table 2: Bleeding disorders in unexplained and explained menorrhagia

N (%)	Unexplained (n=76)	Explained (n=26)	p-value
Bleeding disorder overall	23 (31)	7 (27)	0.48
Von Willebrand Disease	5 (7)	1 (4)	0.58
Low FXI (<70%)	3 (4)	1 (4)	0.73
Other deficiencies *	1 (1)	0	0.75
Platelet defect	16 (21)	7 (27)	0.36

***** factor VII deficiency

Haemostatic variables

Patients had the same PT values as controls (10.9 vs 10.8 sec, p=0.8) but significantly longer aPTT values (mean: 26.5 vs 25.0 sec; p=0.001, table 3), probably caused by lower levels of FXI (mean: 100 vs 124 IU/dL; p<0.001). The levels of coagulation factors IX and XII, which could also influence aPTT values, were comparable in patients and controls, while factor VIII levels tended to be higher in patients (mean: 133 vs 120 IU/dL, p=0.11). VWF:Ag levels were significant lower in patients compared to controls (mean: 91 vs 109; p= 0.024), in line with lower VWF:Rco values, although the latter did not reach statistically significance. Fibrinogen and PI levels were both higher in patients compared to controls. See also table 3.

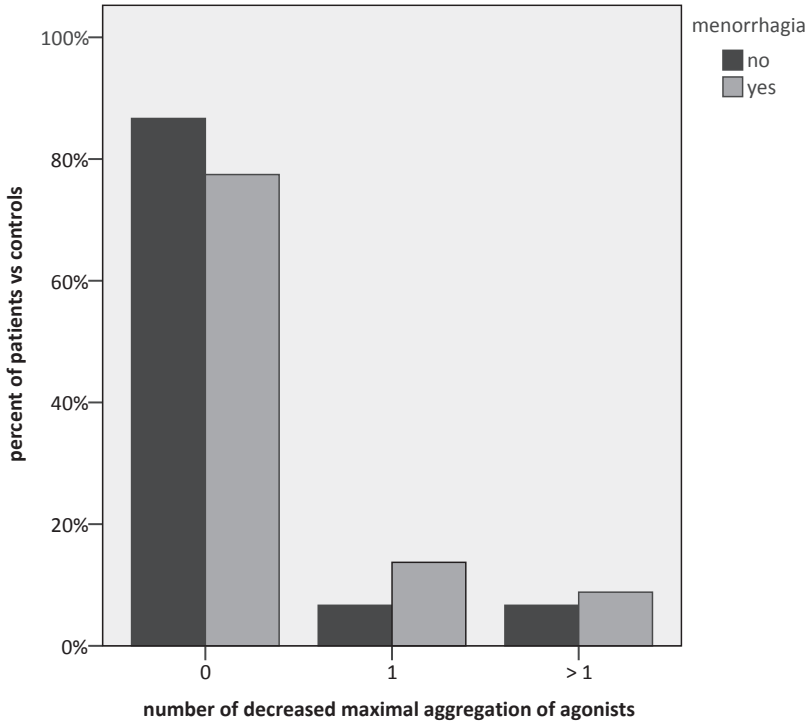
Table 3: Univariate analysis of haemostatic variables in patients vs controls

mean	Patients (n=102)	Controls (n=28)	p-value
PT, sec	10.9	10.8	0.5
aPTT,sec	26.5	25.0	0.001
FVIII:C, IU/dL	133	120	0.11
FIX, IU/dL	110	105	0.24
FXI, IU/dL	100	124	<0.001
FXII, IU/dL	96	98	0.84
vWF:Ag, IU/dL	91	109	0.024
vWF:Rco, IU/dL	94	102	0.19
Fibrinogen, g/L	2.8	2.4	0.002
PI, IU/dL	105	97	<0.001

Platelet disorders

Overall, 23% (23/102) of the patients and 11% (3/28) of the controls had decreased maximal aggregation with one or more agonists (p=0.17). Fourteen patients (14%) and 2 controls (7%) had decreased aggregation with one agonist, 3 (3%) vs 1 (4%) with two agonists, 1 (1%) vs 0 with three agonists, and 5 (5%) vs 0 with four agonists, respectively (p=0.14). See also figure 1. Similar mean maximal aggregation was found in patients vs controls for ristocetin (both 77%); epinephrine (both 80%); collagen (82 vs 80%, respectively) and arachidonic acid (80 vs 82%, respectively). Mean maximal aggregation with 3.3 micromol/mL ADP was higher in patients than in controls (81 vs 77%, p=0.037). We attempted to increase the sensitivity of LTA by using lower ADP concentrations, but this did not distinguish patients from controls: i.e. 1.0 micromol/mL ADP (49 vs 62%, p=0.76), 1.5 micromol/mL ADP (67 vs 64%, p=0.62) and 2.0 micromol/mL ADP (73 vs 71%, p=0.65).

Figure 1: Histogram of number of decreased maximal aggregations (0,1 or >1) with different agonists in patients with menorrhagia (grey) and controls (black)



Bleeding symptoms

Patients experienced significantly more often easy bruising than controls. Overall, 22% of the women with menorrhagia had any bleeding after surgery, tooth extraction or delivery compared to 0%

in controls (p=0.007). The number of bleeding symptoms was the same in women with and without a bleeding disorder (20% vs 22%, respectively, p=0.80). See table 4.

Table 4: Bleeding symptoms and underlying bleeding disorders in patients with menorrhagia and controls

Bleeding symptoms N (%)	Bleeding disorder		p	Menorrhagia		p
	No (n=72)	Yes# (n=30)		No (n=28)	Yes (n=102)	
Menorrhagia since menarche	27 (37)	8 (27)	0.21	0 (0)	35 (34)	<0.001
Epistaxis	11 (15)	1 (3)	0.08	1 (4)	12 (12)	0.18
Easy bruising	18 (25)	8 (27)	0.86	2 (7)	26 (25)	0.027
Excessive bleeding with procedures	2 (3)	3 (10)	0.15	0 (0)	5 (5)	0.30
Post partum haemorrhage (PPH)*	13 (18)	5 (17)	0.60	0 (0)	18 (18)	0.044
Bleeding with tooth extraction	2 (3)	0 (0)	0.51	0	2(3)	0.48
After any surgery, tooth extraction, PPH	16(22)	6(20)	0.80	0	22 (22)	0.007

* only women who had given birth; # FXI <70% vWF:ag or vWF:Rco < 50%, factor VII < 50% or decreased maximal platelet aggregation with ≥1 agonist.

PBAC 100 vs 185

Of the women with menorrhagia, 79 had a PBAC score above 185 vs 4 of the controls. Restricting analyses to patients with a PBAC score above 185 did not change the results. The number of bleeding symptoms in women with and without bleeding disorder was still the same. However, in this subgroup analysis, women with menorrhagia experienced more often easy bruising and a postpartum haemorrhage compared to controls.

Discussion

In this single-centre prospective study we found bleeding disorders in 29% of unselected women with an objectified menorrhagia. We demonstrated that bleeding disorders play an equally important role in the aetiology of both unexplained and explained menorrhagia in this consecutive cohort. The prevalence of bleeding disorders was equally high in menorrhagia with and without gynaecological abnormalities. Women with menorrhagia had significantly lower vWF:Ag and FXI levels than controls. Decreased platelet aggregation was often seen in patients, but also in controls.

Although women with menorrhagia exhibited easier bruising and more bleeding after surgery, tooth extraction or after delivery, non-menorrhagia bleeding symptoms did not predict an underlying bleeding disorder in our cohort.

To our knowledge this is the first cohort that included an unselected cohort of women referred for menorrhagia (PBAC>100). All women had a gynecological examination after which we classified the women as unexplained menorrhagia or due to gynecological abnormalities. All women had haemostatic

testing for underlying bleeding disorders. Moreover, we also included healthy volunteers to compare the haemostatic levels between patients and controls during the 1st week after menstruation.

Our study demonstrated a significantly longer aPTT in patients compared to controls. An increased aPTT with normal PT reflects deficiencies of factors VIII, IX, XI, and/or XII. [14] In our cohort, FIX and FXII were comparable between both groups and FVIII tended to be even higher in patients. Only FXI was significantly lower in patients. Accordingly, the longer aPTT in patients is probably due to lower FXI levels. The clinical relevant lower limit of the normal range for FXI is controversial and varies between 50 IU/dL and 72 IU/dL.[15] Although only a few women had levels below the lower limit of normal, our finding could be relevant as the correlation between FXI concentrations and bleeding symptoms is poor.[15,16] Women with either homozygous or heterozygous FXI deficiency are more likely to have menorrhagia than their unaffected relatives.[16] Previous studies were not designed to find lower but not deficient levels of FXI, as they did not include the appropriate cycle controlled controls.

Patients had higher FVIII and fibrinogen levels compared to controls. This could be the effect of stress due to the anticipation of the gynaecological examination. The prevalence of von Willebrand disease in our cohort is 7% in unexplained menorrhagia and 4% in women with menorrhagia due to gynaecological abnormalities. The prevalence in women with unexplained menorrhagia is comparable to some other (American) studies,[9,17] but most studies reported a higher prevalence.[8,10] Ethnicity might be one factor, as our cohort was almost exclusively Caucasian. The diagnosis of VWD is complex and the diagnosis of mild forms can be difficult.[18] Furthermore, we performed in our cohort haemostatic evaluation in the early follicular phase (1st week after menstruation), because of fluctuation of haemostatic variables during menstrual cycle. In a previous systematic review,[11] which summarizes the evidence that haemostatic variables vary during the menstrual cycle, we reported that vWF and FVIII:C had their lowest levels during the menstrual and early follicular phase. Most other studies which evaluated the prevalence of underlying bleeding disorders in menorrhagia patients tested randomly and took no account of cyclic variations.[7, 8, 17, 19] Accordingly, we expected that the prevalence of VWD in our cohort is not underestimated, because by measuring in the 1st week after menstruating, we increased the sensitivity of finding an underlying bleeding disorder as VWD.[20-22] Furthermore, vWF:Ag was significantly lower in patients compared to the controls. This could not be explained by the blood group O effect, because this was equally divided in both groups.

The number of defects in platelet aggregrometry seems to be comparable between patients and controls (23 vs 11%; p=0.17), but the number of decreased maximal aggregations with different agonists per women was higher in patients (Figure 1). It is possible that statistical significance was not attained in our cohort due to the small numbers of controls we had involved. Also the prevalence in women with unexplained and explained menorrhagia seems to be comparable. Only one other study by Philipp et al[23] evaluated the prevalence of platelet aggregation defects specifically. They reported in 47% a decreased aggregation with one or more agonists in women with menorrhagia, which is much higher compared to our cohort. One possible explanation for the different prevalence rates could be the test itself. Laboratory tests for platelet function disorders are not well standardized due to test

complexity and the need to rapidly process freshly collected blood samples.[24]

We cannot identify a specific or combination of non-menorrhagia bleeding symptoms as a predictor for an underlying bleeding disorder in our cohort. Some other studies suggest that symptoms, like bleeding after tooth extraction, post-operative bleeding and postpartum bleeding, may be more valuable to predict an inherited bleeding disorder.[25] Although in our study patients with menorrhagia more often had additional bleeding symptoms than controls, there was no difference between patients with and without bleeding disorders. We suggest that clinicians consider haemostatic evaluation in patients with menorrhagia, independent of presence or absence of other bleeding symptoms or gynaecological abnormalities.

Our study has some limitations. First, we performed the platelet aggregometry only once in all patients and controls. This is supported by Quiroga et al,[26] who reported that most repeated tests by LTA confirm initial findings. Nonetheless, repeat testing is recommended to rule out pre-analytical or analytical false positive abnormalities. Second, a proportion of the controls had also a PBAC score above 100 although we only included healthy volunteers with a subjective normal menstruation. Apparently, not all Dutch women with heavy menstruation consider their menstruation pattern abnormal. This is also known from the literature, the correlation between the observed PBAC score and the subjective menstrual blood loss is poor.[27]

Our study confirms the results of other studies that haemostatic disorders play an important role in the aetiology of menorrhagia. A new finding is that this is also true in women with concomitant gynaecological abnormalities. To our knowledge this is the first reported study that found significantly lower levels of FXI in women with menorrhagia compared to controls. This together with its clinical relevance must be confirmed in a larger cohort of women with menorrhagia.

Recognizing and diagnosing underlying bleeding disorders like VWD in women with menorrhagia can have important clinical implications because it enables effective medical treatment of menorrhagia with desmopressin nasal spray and antifibrinolytic agents in addition to oral contraceptive pill[28] used alone or in combination, and recognition of increased bleeding risk during major surgical intervention. Further, most underlying bleeding disorders like VWD are inherited disorders, which could lead to screening of family members when a case is identified.

In conclusion, underlying bleeding disorders play an important role in the aetiology of unexplained as well as menorrhagia with gynaecological abnormalities. Haemostatic testing must be considered, independently of other bleeding symptoms and gynaecological abnormalities.

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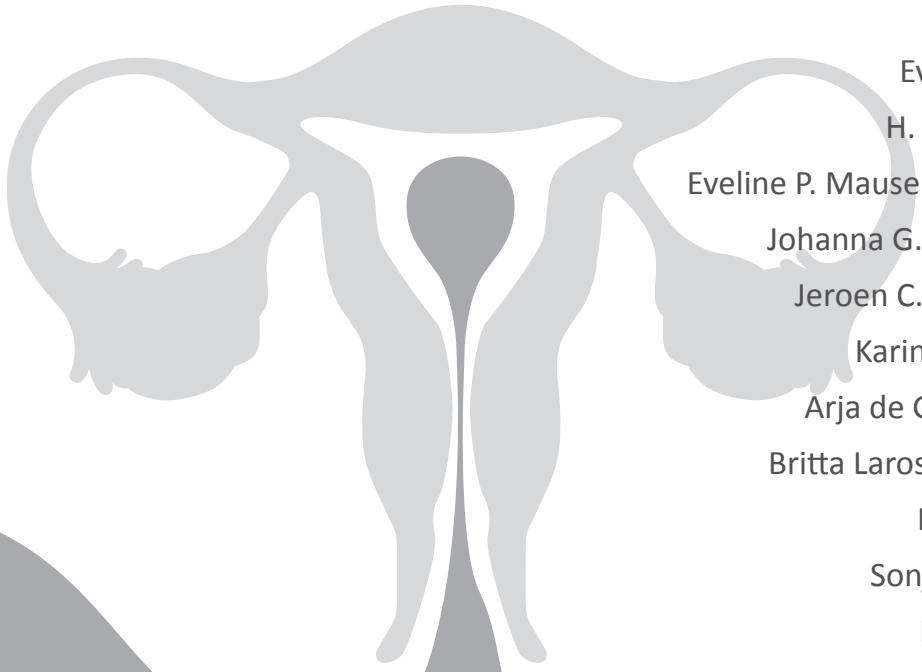
Chapter 4

Gynaecological and obstetric bleeding in moderate and severe Von Willebrand Disease



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Abstract

A nation-wide cross-sectional study was initiated to assess gynaecological and obstetrical symptoms in an unselected cohort of women with moderate and severe VWD in the Netherlands. 423 women aged ≥16 years were included. Bleeding severity was measured using the Tosetto Bleeding Score (BS). Menorrhagia, defined as occurrence of ≥2 menorrhagia symptoms, was reported by 81%. Of all VWD women, 78% received any kind of treatment for menorrhagia and 20% underwent a hysterectomy predominantly because of severe menstrual bleeding. Over half of the women reported more blood loss than can be expected with a normal delivery. In 52% of reported pregnancy losses curettage was needed because of bleeding. Mean number of live births was 1.9, which is comparable with the general Dutch population.

In conclusion, women with moderate or severe VWD frequently have menorrhagia in need of treatment and 20% of the VWD women underwent a hysterectomy. Bleeding complications occurred in over 50% of the women after childbirth or pregnancy loss. Progeny seems not to be affected in women with moderate or severe VWD.

Introduction

Von Willebrand Disease (VWD) is caused by defects in or reduced levels of Von Willebrand Factor (VWF). It is the most common inherited bleeding disorder and affects 0.5-1% of the population, although not all patients with low VWF levels have clinically relevant bleeding episodes.[1, 2]

Patients with VWD frequently have bleeding episodes, varying from gum bleeds and epistaxis to intestinal bleeding. In theory, men and women are equally likely to be affected, but in women VWD is more often clinically manifest because of the bleeding challenges that are associated with menstruation and childbirth.[1] Tosetto et al. have developed a bleeding score (BS) to quantify the number and severity of bleeding symptoms.[2] Two of the 12 items of the BS include menorrhagia and postpartum haemorrhage. We used the BS, a validated and commonly used instrument, to determine the severity of menorrhagia and postpartum haemorrhage.

The majority of published studies investigating the prevalence of gynaecological symptoms in women with VWD are case series of a relatively small number of women.[3-7] In addition these women had predominantly type 1 or mild VWD. In these studies women with VWD frequently have menorrhagia with reported prevalence ranging from 74-92%, which may impair quality of life (QoL).[4,5,8] Also increased absence from school or work during menstruation is reported.[5,6,9-11] One study reported that women with VWD more often underwent hysterectomy than women without VWD.[12] The above mentioned studies may suffer from selection bias given the fact that patients seeking medical attention for bleeding and menorrhagia have predominantly been included.

Therefore the aim of our study was to assess gynaecological and obstetrical symptoms in a large unselected cohort of women with moderate or severe VWD who participated in a nation-wide study.

Methods

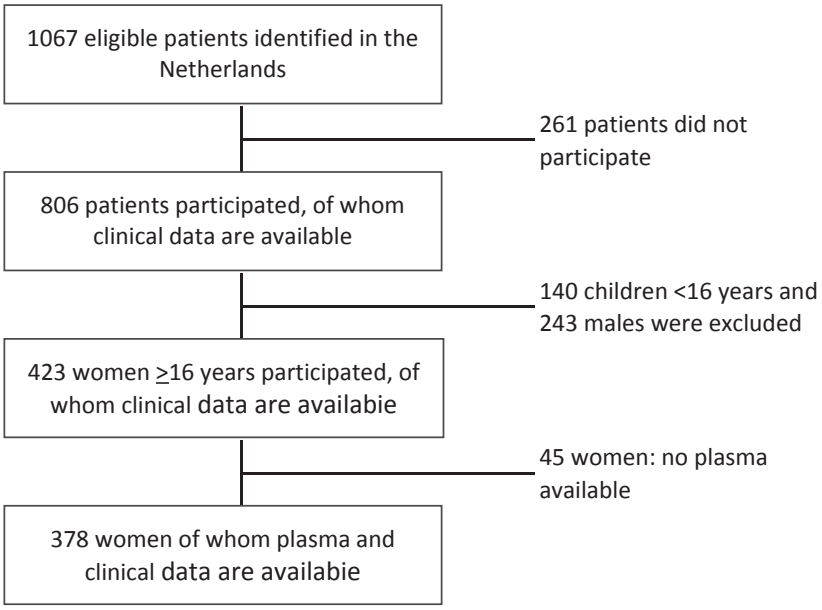
Participants

We performed a nation-wide cross-sectional study among patients with moderate and severe VWD in the Netherlands, the “Willebrand in the Netherlands” (WiN) study. Data on gynaecological and obstetric bleeding were obtained retrospectively. Patients were recruited at all 13 Hemophilia Treatment Centers (HTCs) in the Netherlands. We included patients diagnosed with type 1, type 2 and type 3 VWD who fulfilled both of the following inclusion criteria: 1) hemorrhagic symptoms or a family history of von Willebrand disease; 2) historic levels of VWF antigen (VWF:Ag) ≤ 30 U/dL and/or VWF activity (VWF ristocetin cofactor activity (VWF:RCo) and/or VWF collagen binding assay (VWF:CB)) ≤ 30 U/dL and/or FVIII:C ≤ 40 U/dL at least once. Classification of VWD into type 1, 2 and 3 was based on VWF parameters measured in laboratories of the various HTCs and according to classification guidelines.[9,10] Patients with mild VWD were excluded, as were patients with other congenital disorders of hemostasis resulting in a hemorrhagic diathesis.

For the present analyses we selected all women aged 16 years and older. Data were obtained

between October 2007 and October 2009. The Medical Ethical Committees at all participating HTCs approved this study, and written informed consent was obtained from all study participants.

Figure 1: Flow chart of study inclusion.



Assessment methods

All participants completed an extensive questionnaire, which contained questions on bleeding episodes, treatment of VWD, side effects of treatment, concomitant disease, and employment.[13] The Bleeding Score was incorporated into this questionnaire.

The Bleeding Score was used as previously described for bleeding severity in type 1 VWD by Tosetto et al.[2] It systematically evaluates bleeding symptoms, and accounts for both the number and severity of the bleeding symptoms. The severity and frequency of 12 items are scored on a scale ranging from -1 to 4 points. Higher scores reflect more severe/frequent bleeding. The total for all 12 items results in a Bleeding Score (range 3 to 45).

Definitions

Severe VWD was defined as the presence of at least one of the following laboratory abnormalities: VWF:Ag ≤ 10 U/dL, and/or VWF:RCo ≤ 10 U/dL, and/or FVIII:C ≤ 20 U/dL. Moderate VWD was defined as VWF:Ag 10-30 U/dL, and/or VWF:RCo 10-30 U/dL, and/or FVIII:C 20-40 U/dL.[11]

Menorrhagia was defined as the occurrence of at least two of the symptoms listed in table 1A.[14-16] Severity of menorrhagia was determined according to the menorrhagia items of the Tosetto Bleeding

Score (BSmenorrhagia).[2] The score for this item ranges from 0 to 4 (table 1B).

Severity of bleeding complications following childbirth was determined according to the postpartum haemorrhage (PPH) item of the Tosetto Bleeding Score (BS-PPH).[2] The score for this item ranges from -1 to 4 (table 1B).

Fetal loss was defined as spontaneous miscarriages, fetal death and intrauterine death.

Table 1: A) Definition of menorrhagia and B) classification of severity of menorrhagia and postpartum haemorrhage, according to the Tosetto Bleeding Score

A) Menorrhagia: ≥2 symptoms at the time of study or in the past
• subjective excessive menstrual bleeding
• loss of blood clots during menstrual bleeding
• requirement of oral iron therapy or blood transfusion
• heavy menstrual flow that interferes with daily life
• menstrual period that lasts longer than 7 days
B) Severity of bleeding symptoms*
Severity of menorrhagia (BSmenorrhagia)
Score 0: No menorrhagia
Score 1: Consultation only
Score 2: Antifibrinolytics or pill use
Score 3: Dilatation and curettage or iron therapy
Score 4: Blood transfusion, FVIII/VWF concentrate, desmopressin or hysterectomy
Severity of PPH (BS-PPH)*
Score -1: No bleeding in at least two deliveries
Score 0: No deliveries or no bleeding in one delivery
Score 1: Consultation only
Score 2: Dilatation and curettage, iron therapy, antifibrinolytics
Score 3: Blood transfusion or FVIII/VWF concentrate or desmopressin
Score 4: Hysterectomy

* BS=Bleeding Score, scores are derived from the Tosetto Bleeding Score[18]

Laboratory measurements of VWD

Historic measured VWF and FVIII levels in the Hemophilia Treatment Centers were used as inclusion criteria for the WiN study, and patients with at least one measurement of VWF or FVIII below 30 U/dL or 40 U/dL respectively were included.

Peripheral venous blood was collected at inclusion of the study. Plasma levels of VWF antigen (VWF:Ag), VWF Collagen Binding (VWF:CB), VWF activity (VWF:Act) and FVIII activity (FVIII:C) were measured centrally in the Erasmus university Medical Center, Rotterdam, The Netherlands. VWF:Ag level was measured with an in-house ELISA using a polyclonal rabbit anti-human VWF antibody

(DakoCytomation, Glostrop, Denmark) for capturing and a HRP-conjugated anti-human VWF antibody (DakoCytomation, Glostrop, Denmark) for detecting. VWF:CB level was measured with an in-house ELISA using collagen type 1 (Sigma-Aldrich, St Louis, USA) for capturing and a HRP-conjugated anti-human VWF antibody (DakoCytomation, Glostrop, Denmark) for detecting. To assess VWF activity we have used an VWF:Act assay that measures the ability of VWF to bind GpIbα. The VWF:Act assay uses latex particles coated with a monoclonal murine antibody direct against the GpIbα binding domain of VWF (Instrumentation Laboratory B.V, Breda, The Netherlands). These latex particles were incubated with the patient plasma and agglutination of the particles, proportionally to the GpIbα binding activity of VWF, was measured.[17] In the Erasmus university Medical Center Rotterdam we have validated this test and compared it with the VWF:RCo activity test. We obtained a Spearman correlation coefficient of 0.942 FVIII:C was measured in a one-stage clotting assay (TriniCLOT, Biomerieux, Marcy l'Etoile, France) with FVIII-deficient plasma (Biopool, Umea, Sweden). Multimeric pattern was evaluated by low resolution 0.9% agarose (Bio-Rad Laboratories, Hercules, CA, USA) gel electrophoresis followed by capillary Western blotting.[13] VWF multimer patterns were evaluated by two independent reviewers (HCJE and FWGL). VWF multimers were classified as either abnormal, normal or absent by comparison with the commercial reference plasma (Normal reference plasma, Precision biologic, Kordia, Leiden, Netherlands). Abnormal multimers were defined as a deviation from a normal distribution; according to the MCMDM-1VWD study.[18]

Determination of type of VWD into type 1, type 2 and type 3 VWD and subclassification was based on the centrally determined VWF and FVIII parameters, according to ISTH guidelines.[9, 10, 19]

Statistical methods

Continuous variables, expressed as medians (ranges) were used for age of menarche, duration of period, number of days of heavy menstrual bleeding, and data on deliveries and PPH. The chi-square test was performed to analyse differences between the prevalence of symptoms and bleeding scores between subgroups. ANOVA test was used to analyse differences in age and duration of menstrual bleeding, and for data on deliveries and PPH. Significant differences were defined as a p-value ≤0.05.

Results

A total of 423 women were included in the study, see figure 1. Table 2 represents the patient characteristics. Median age was 46 (range 16-83) years. A total of 242 (64%) women had type 1 VWD, 120 (32%) had type 2 VWD, and 16 (4%) had type 3 VWD.

Table 2: Patient characteristics of the women included in the study (n=423)

age	median, range	46	16-83
VWD type*	1 (n,%)	242	64%
	2 (n,%)	120	32%
	2A	83	
	2B	15	
	2M	14	
	2N	8	
	3 (n,%)	16	4%
VWF:Ag*	median U/dL, IQR	33	21 to 47
VWF:CB*	median U/dL, IQR	28	11 to 57
VWF:Act*	median U/dL, IQR	28	12 to 59
FVIII:C*	median U/dL, IQR	56	37 to 80
VWD severity*	severe VWD	121	32%
	moderate VWD	257	68%
Bleeding Score	median, range	12	-1 to 35

*n=378 based on patients of whom plasma was available
IQR: interquartile range

Menorrhagia in women with moderate or severe VWD

Median age of menarche was 13 years (interquartile range 12-14), table 3. The median duration of menstrual bleeding of the women included in this study was 7 days. Menorrhagia, defined as occurrence of ≥2 menorrhagia symptoms, was reported by 81% of the women (table 3). The two most frequent symptoms were excessive menstrual bleeding (82%) and loss of blood clots (80%). No differences were observed for type 1, type 2 and type 3 VWD.

Eighty-five percent of all VWD women had consulted their general practitioner or gynaecologist because of menorrhagia, including 24 women who did not qualify as menorrhagia according to our strict definition.

Treatment of menorrhagia in patients with moderate or severe VWD

Nearly all women with menorrhagia (99%) had used some treatment for menorrhagia. Figure 2 shows which treatment women with moderate or severe VWD received because of menorrhagia. Most women with menorrhagia use or have used hormonal contraceptives (hormone therapy, oral contraceptives, or levonorgestrel intrauterine device) to control menstrual blood loss (68%). In 10% of the women desmopressin was ever given because of menorrhagia. Eleven percent of the women received a blood transfusion at least once because of anaemia due to menorrhagia. Fifty-six percent of the women with type 3 VWD received a blood transfusion, FVIII/VWF concentrate, or underwent a hysterectomy because of menorrhagia.

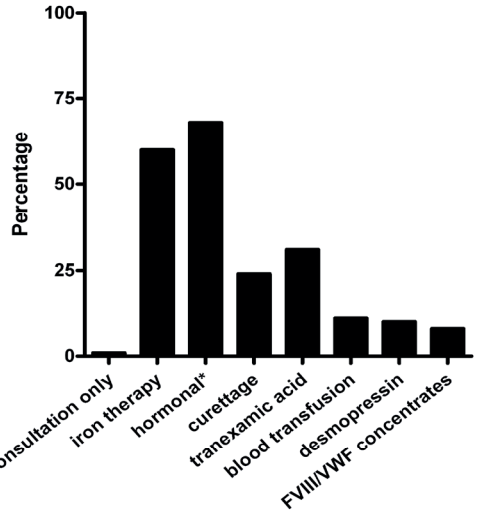
Table 3: Menorrhagia in women with moderate or severe VWD

		total		type 1		type 2		type 3		
		n=423		n=242		n=120		n=16		p*
age of menarche	median, IQR	13	12-14	13	12-14	13	12-14	13	12-14	0,63
duration period	median, IQR	7	6-8	7	5-8	7	6-8	7	7-10	0,76
number of days heavy menstrual bleeding	median, IQR	4	3-5	4	3-6	4	3-5	4	3-6.5	0,41
Menorrhagia symptoms	median, IQR	3	2-4	3	2-4	3	2-4	3	1-4	0,52
excessive menstrual bleeding	n, %	347	82%	202	83%	98	82%	13	81%	0,19
loss of blood clots	n, %	338	80%	196	81%	100	83%	10	63%	0,48
requirement of iron or blood transfusion	n, %	184	44%	105	43%	60	50%	10	63%	0,08
heavy menstrual flow that interferes with daily life	n, %	192	45%	110	45%	58	48%	7	44%	0,77
menstrual period that lasts > 7 days	n, %	150	36%	81	33%	51	43%	6	38%	0,16
menorrhagia ≥ 2 symptoms present	n, %	342	81%	196	81%	99	83%	12	75%	0,76
Menorrhagia severity	median, IQR	3	2-4	3	2-4	3	2-4	3	2-4	0,10
0: no menorrhagia	n, %	64	15%	41	17%	13	11%	1	6%	
1: consultation only	n, %	29	7%	19	8%	5	4%	1	6%	
2: antifibrinolytics or pill use	n, %	98	23%	46	19%	31	26%	5	31%	
3: dilatation and currettage or iron therapy	n, %	121	29%	74	31%	37	31%	3	19%	
4: blood transfusion, FVIII/VWF concentrate, desmopressin or hysterectomy	n, %	111	26%	62	26%	34	28%	9	56%	

* Chi square or ANOVA test for differences between subgroups
IQR: interquartile range

Of all included women with VWD, 20% (n=84) underwent a hysterectomy. In the group of women >40 years 28% underwent a hysterectomy. Of the women with type 1, type 2, and type 3 VWD respectively 24%, 14% and 13% underwent a hysterectomy. In 31 (37%) of the women VWD was diagnosed after the hysterectomy, in 29 (35%) before hysterectomy, whereas in the other women this was unknown. The median age at the time of hysterectomy was 37 years (range 26-54). Median age at the time of hysterectomy did not differ for women who were diagnosed before (38, range 27-51) or after (37, range 26-53) the hysterectomy, p= 0.358. Data on surgery-related bleeding was available in 50 hysterectomies, of which 29 were complicated by a bleeding (58%). A hysterectomy was more often complicated by bleeding if VWD was not yet diagnosed before the hysterectomy. A hysterectomy was performed in 68% of the women because of menorrhagia. In the other women it is unknown whether bleeding was the cause of the hysterectomy. Two women underwent endometrial ablation.

Figure 2: Treatment of menorrhagia in women with moderate or severe VWD (n=342).



* hormone therapy, oral contraceptives, hormone releasing IUD patients may have used more than one treatment option

Pregnancies and bleeding in patients with moderate or severe VWD

Of the total cohort of 423 women 314 (74%) had ever been pregnant. The mean number of live births per woman with moderate or severe VWD above the age of 40 years is 1.9. The 314 women had 691 deliveries. Of the 314 women 159 (51%) reported more blood loss than can be expected with a normal delivery, see table 4. This was not different in women who gave birth recently or decades ago. In women aged 16-40 years, 41 to 60 years and >60 years a PPH occurred in 46%, 51% and 55% respectively (p=0.610). In 77 (11%) of the 691 deliveries a blood transfusion was given because of postpartum haemorrhage (PPH).

Table 4: Bleeding complications during deliveries in women with VWD who have been pregnant at least once

		total n=314		moderate VWD n=221*		severeVWD n=62*		p-value
Number of deliveries	n	691		513		116		0,006
Number of deliveries with postpartum hemorrhage	n, %	258	37%	181	35%	51	44%	0,168
Number of women with postpartum hemorrhage	n, %	159	51%	107	48%	35	56%	0,263
Tosetto bleeding score postpartum hemorrhage								0,046
-1: no bleeding in at least two deliveries	n, %	121	39%	93	42%	18	29%	
0: no deliveries or no bleeding in one delivery	n, %	43	14%	28	13%	8	13%	
1: consultation only	n, %	49	16%	38	17%	7	11%	
2: dilatation and curettage, iron therapy, antifibrinolytics	n, %	8	3%	5	2%	1	2%	
3: blood transfusion or FVIII/VWF concentrate or desmopressin	n, %	90	29%	54	24%	28	45%	
4: hysterectomy	n, %	3	1%	3	1%	0	0%	

* Chi square or ANOVA test for differences between subgroups
*n=283 based on patients of whom plasma was available

Twenty-seven percent of the primary PPHs (within 24 hours after childbirth) occurred in women who received prophylactic FVIII/VWF concentrate or desmopressin. The severity of PPH is reflected in Tosetto bleeding score on PPH (BS-PPH). Of the 314 women who have been pregnant, 101 (33%) had a BS-PPH of ≥2, indicating that they were treated for PPH. A blood transfusion, FVIII/VWF concentrate or desmopressin was needed in 90/314 (29%) women, and 3 women (1%) underwent a hysterectomy because of a massive PPH.

BS-PPH was significantly different between women with moderate and severe VWD: women with moderate VWD had a median BS-PPH of 0 (interquartile range -1 to 4), whereas women with severe VWD had a median BS-PPH of 1 (range -1 to 3) (p=0.046). We found an association between VWF levels, FVIII level and BS-PPH. Patients with the lowest VWF and FVIII levels had the highest BS for the item PPH. Women with VWF:Ag, VWF:Act or FVIII levels <10 IU/dL compared to women with levels ≥10 IU/dL, had a 1.0 (0.3-1.7), 0.5 (0.0-0.9), and 1.8 (0.6-3.0) point BS-PPH increase respectively.

Spontaneous abortion, fetal death and intrauterine death in moderate or severe VWD

We collected data on elective abortions, spontaneous miscarriages and fetal deaths. Twenty women did not fill in this part of the questionnaire, therefore data were available for 294 of the 314 women who have been pregnant. Of these, 115 women (39%) had a total of 201 pregnancy losses (elective abortions, spontaneous miscarriages and fetal deaths). In 52% of the pregnancy losses curettage was needed because of bleeding.

Discussion

In this nationwide study of a large unselected cohort of 423 women with moderate to severe VWD in the Netherlands, we have demonstrated a very high prevalence of menorrhagia. Nearly 80% of all the VWD women had used medication or underwent an intervention because of menorrhagia. Of all included women with VWD, 20% underwent a hysterectomy. Moreover, a high incidence of bleeding complications associated with pregnancies was observed. Thirty-nine percent of these women had a pregnancy loss, half of the women needed curettage because of bleeding after pregnancy losses. Progeny was comparable with the general Dutch population.

In this largest cohort of women with VWD described so far, including the majority of all women with moderate to severe VWD in the Netherlands, 81% reported the occurrence of 2 or more symptoms of menorrhagia and 85% sought medical attention for menorrhagia. This is comparable with other, smaller studies about menorrhagia in women with VWD.[4,5,8,20] These are very high numbers in comparison to the general population in which 5-10% of women in reproductive age has sought medical attention for menorrhagia.[21] There is a discrepancy between the percentage of women consulting a physician for menorrhagia (85%) and the percentage of women with menorrhagia (81%). Probably most of the women have menorrhagia, but did not fulfil the strict definition of menorrhagia we used in our study. In addition, women may subjectively perceive their menses as normal when

mothers or sisters also have menorrhagia.

The high number of hysterectomies (20%) in our VWD cohort is of particular concern. The proportion of hysterectomies is nearly twice as high as previously reported in a Dutch study on women with chronic menorrhagia. In this study 11% of the women underwent a hysterectomy at a median age of 42.[22] The women in our study underwent the hysterectomy at a younger age (median 38 years), which is comparable with other studies on women with VWD.[4, 6, 8, 20] A hysterectomy was complicated by bleeding more often if VWD was not yet diagnosed. It is therefore of utmost importance that gynaecologists consider inherited bleeding abnormalities including VWD, because in these women other treatment options, i.e. intranasal desmopressin and/or tranexamic acid, might have resulted in less menstrual blood loss. In case surgery is still needed, desmopressin or FVIII/VWF concentrate can be given perioperative to prevent bleeding complications. Fortunately the high proportion of hysterectomies does not seem to reduce the progeny, as the number of children is similar to the general population.

Despite the increased awareness of bleeding problems in women with VWD in the last decades,[14,23,24] postpartum haemorrhage is still a major concern in these women. Treatment options like FVIII/VWF concentrate became available, nevertheless we observed no reduction in postpartum haemorrhage over the last decades. A blood transfusion after delivery was more often needed in VWD women (11% of all deliveries) compared to the general population in which the incidence of blood transfusion after vaginal delivery and caesarean section is 1% and 1-7%, respectively.[25] In our study we used retrospective data, therefore it is impossible to definitely confirm the diagnosis of postpartum haemorrhage, furthermore it is known that surveys on postpartum haemorrhage show higher numbers than discharge summaries.[26] Remarkable, improvement of care and guidelines has not decreased the frequency of postpartum haemorrhage. The cause of postpartum haemorrhage in women who received prophylactic treatment was unknown. Prospective studies are needed in order to improve outcome and to optimize current treatment guidelines.[27]

A main concern of many women with VWD is whether they have lower rates of conception or a higher chance of miscarriages or spontaneous abortions. Our study revealed a mean number of live births of 1.9 per woman above the age of 40, which is comparable with the general Dutch population (1.8).[28] Therefore, we concluded that having VWD does not result in fewer children. Our questionnaire did not distinguish between early and late fetal loss, therefore it is not possible to draw firm conclusions about the prevalence of fetal loss in our cohort. A very high number of women (52%) needed curettage because of bleeding after pregnancy losses. This is in line with a previous study, in which also a high incidence of post-abortal bleeding was observed.[29] In the general population the number of curettage after pregnancy loss was only 2-20%.[30] The high number of bleeding can partly be explained by the low FVIII and VWF levels in VWD, which do not rise significantly until the second trimester by which stage many fetal losses have already occurred.[31, 32]

Our study has some limitations. First the study design, we performed a retrospective study in which data were gathered using a self-completed questionnaire, and we have only self-reported data about

menorrhagia and postpartum haemorrhage. Recall bias may be a potential problem. We determined the severity of menorrhagia and PPH using the Tosetto Bleeding Score and did not quantify blood loss for instance with the pictorial blood loss assessment chart (PBAC) score. However data on the need of blood transfusion seem to be reliable and showed an increase in the VWD women compared to the general population. We defined menorrhagia as the presence of ≥ 2 symptoms (see table 1), based on available literature and recommendation of an international expert panel.[14-16] Second, the Tosetto Bleeding Score used in this study was designed as a physician-administrated questionnaire and not for self-administration. However, patients with severe VWD and type 3 had higher bleeding scores, compared to patients with type 1 VWD and moderate VWD, suggesting that this self-completed questionnaire revealed reliable results. A third limitation is that the Tosetto Bleeding Score was originally developed only to distinguish between adult type 1 VWD patients and patients without VWD.[2, 33] However recently also others have successfully used the Bleeding Score in type 2 and 3 VWD,[34,35] and we think that this also reflects severity of bleeding phenotype in our cohort of patients.

The strength of our study is the large number of unselected women included. Our study covers almost all women with moderate and severe VWD in the Netherlands, since the large majority of all individuals who were diagnosed with moderate or severe VWD in any of the 13 Dutch Haemophilia Centres participated in the study. Therefore referral bias is limited, especially since our cross-sectional study included all women, regardless of the presence of menorrhagia. Finally, central laboratory testing of VWF and FVIII levels was performed, excluding bias by inter-laboratory differences.

In conclusion, women with moderate or severe VWD frequently have menorrhagia in need of treatment and a large proportion of the VWD women underwent a hysterectomy. Bleeding complications occur in over half of the women after childbirth or pregnancy loss. Progeny seems not to be affected in women with moderate or severe VWD.

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Conflict of Interest Disclosures

FWGL is a member of the haemophilia advisory board of CSL Behring, and attended a round table meeting of Baxter. JCJE received research support from CSL Behring. KM and EPMB are members of the haemophilia advisory board of CSL Behring. J.G. van der Bom has received unrestricted research/educational funding for various projects from the following companies: Bayer Schering Pharma, Baxter, ZLB Behring, Novo Nordisk, and Wyeth. In addition, she has been a consultant to Baxter and Wyeth, and she has been a teacher on educational activities of Bayer Schering Pharma. None of the other authors has a conflict of interest to declare.

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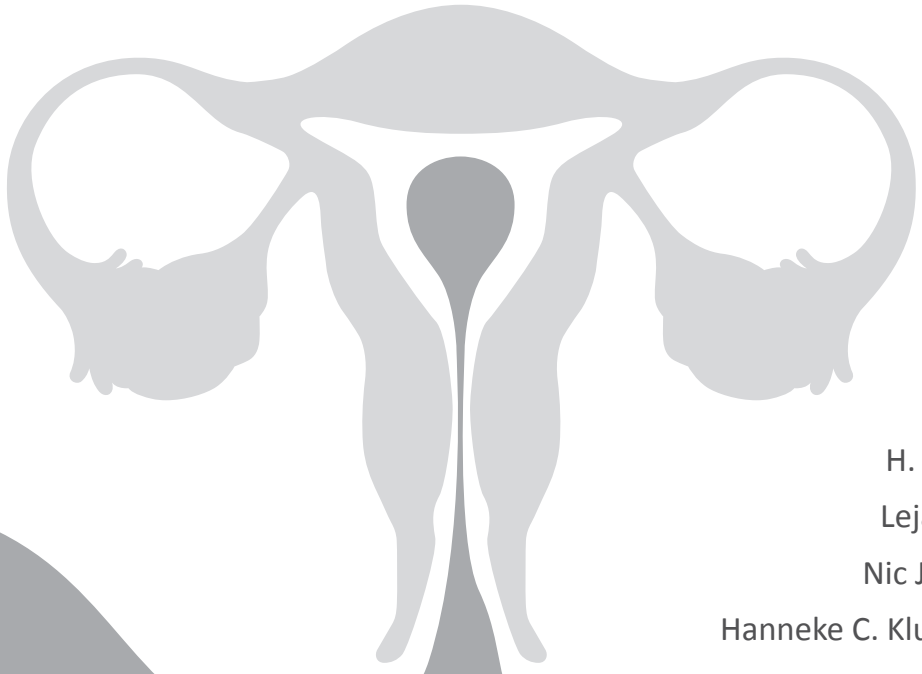
Part II

Bleeding issues in obstetrics

Chapter 5

The risk of postpartum hemorrhage in women using high dose of low-molecular-weight heparins during pregnancy



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Abstract

Background: Low-molecular-weight heparins (LMWH) are the most commonly used anticoagulant during pregnancy for prevention or treatment of VTE. However, the size of the associated risk of postpartum haemorrhage (PPH) is unknown.

Objective: to assess the bleeding risk of high dose LMWH, also in relation to the time between last dose LMWH and delivery.

Material and methods: from 1999 to 2009, we followed 88 pregnant women who were started on therapeutic anticoagulation. Controls were pregnant women without LMWH, matched 1:4 for parity, mode of delivery, age, gestational age and delivery date. PPH was defined as ≥500 ml blood loss for vaginal delivery (severe PPH in vaginal delivery as ≥1000 ml) and ≥1000 ml for cesarean section (CS). Women were divided into subgroups by the interval between last dose of anticoagulation and delivery (<12, 12-24hrs, >24hrs).

Results: Risk of PPH after vaginal delivery was 30% and 18% for LMWH-users and non-users, respectively (OR 1.9, 95%CI 1.1-3.5). Risk of severe PPH after vaginal delivery was not different (5.6 vs 5.0%; OR 1.1; 0.4-3.6). Risk of PPH after CS was 12% in LMWH-users and 4% in non-users (OR 2.9; 0.5-19.4). Both events of LMWH-users occurred after emergency CS. The risk of PPH associated with delivery within 24 hours after last dose of LMWH was 1.2 fold higher (95%CI 0.4-3.6) compared to a larger interval.

Conclusion: high dose LMWH carries an increased risk of more than 500 mL blood loss after vaginal delivery. However, this results not in more clinical relevant severe PPHs. The interval between last dose of LMWH and delivery does not influence the risk of PPH.

Introduction

In the general population, 0.5 in 1000 pregnancies[1] is complicated by a venous thrombo-embolism (VTE), with a predominance in the puerperium.[2,3] In women with a previous episode of VTE, the risk of recurrence during pregnancy ranges from 2.4-6.2%.[4,5] For these women, with either a current VTE or a high risk of recurrent VTE, low molecular weight heparin (LMWH) is the most commonly used anticoagulant during pregnancy. The optimal dosage of thromboprophylaxis in women with an increased risk of VTE during pregnancy and puerperium is not established.[6] In our hospital, all pregnant women with an indication for thromboprophylaxis received a high dose intended as a therapeutic dosage of LMWH during pregnancy and the puerperium.

Usage of LMWH during pregnancy may be associated with an increased risk of blood loss or postpartum hemorrhage (PPH), a common complication of childbirth and a leading cause of maternal morbidity and mortality. Few studies assessed the risk of PPH associated with usage of LMWH,[7-14] but most studies are retrospective cohort studies, without a control group and describing only a small number of women on therapeutic dosage LMWH.

Further, current guidelines recommend discontinuing LMWH at least 24 hours before labor,[6] although no data are available whether this influences the risk of PPH. This is challenging as labor may start unplanned.

We performed a retrospective cohort study in our hospital to evaluate our treatment protocol to assess the bleeding risk with high dose therapeutic dosage LMWH during delivery compared to controls and to assess the bleeding risk in relation to the last injection of LMWH.

Methods

Patients

This is a retrospective cohort study, including as cases consecutive women who started with a therapeutic dosage LMWH during pregnancy at our hospital between 1999 and 2009. These women were followed prospectively during pregnancy in a combined obstetric/ coagulation clinic and seen by a thrombosis specialist every 2 months until 6 weeks post-partum. Indications for anticoagulation were a history of idiopathic, provoked or pregnancy related VTE, recurrent fetal loss or asymptomatic thrombophilic defects (protein C, S or AT deficiency). For a given woman, we included only the first ongoing pregnancy in which she used anticoagulation, to avoid selection bias. Pregnancies with early fetal loss were not included. Detailed information on episodes of VTE, external risk factors for thrombosis, obstetric history, anticoagulant treatment and delivery was collected using a standardised questionnaire. Data on labor was collected retrospectively by reviewing medical records. National legislation and the ethical committee of our institution approve this type of studies without the need for review of the protocol.

Treatment protocol

Women were started on a therapeutic dosage of nadroparin once daily in early pregnancy with bodyweight adjusted therapeutic dosage (175 anti-Xa IU kg^{-1} day^{-1}), as soon as a pregnancy test was positive or when a VTE occurred during pregnancy. All women were followed by a thrombosis specialist every 2 months until 6 weeks post-partum. Anti-fXa levels were not routinely monitored and doses of LMWH were not adjusted for increasing bodyweight or increasing renal clearance. If hypersensitivity skin reactions developed, we switched to another preparation, fondaparinux, danaparoid or acenocoumerol. The women had no planned induction of labor with withholding of anticoagulation, but all women switched to divided (twice daily) dosing of their LMWH in the 37th week to minimize the bleeding risk. Women who used acenocoumerol switched to a twice daily dosing LMWH in the 37th week. Second, LMWH or another preparation was stopped at the start of spontaneous or induced labor and restarted 4-8 hours after delivery (when blood loss was normal) and stopped six weeks postpartum. Women with a current VTE during pregnancy were treated for six months, but at least until six weeks postpartum.

Controls

Controls were women who delivered in our hospital and did not use LMWH or another anticoagulant during pregnancy. LMWH users were matched 1:4 to controls (non-users) by random electronic selection for parity, mode of delivery, age, gestational age, and date of delivery (+/- 2 years). Exclusion criteria for the controls were a history of VTE or PPH.

Definitions

The amount of blood loss was a visual estimation. According to the WHO guidelines, we defined $\geq$500 ml as definition of PPH for vaginal delivery and $\geq$1000 ml for a cesarean section. Severe PPH was defined as $\geq$1000 ml for a vaginal delivery. Primary and secondary PPH were defined as a bleeding within 24 hours after delivery and after 24 hours, respectively.[15]

Statistical analysis

Continuous variables were expressed as mean or median values and standard deviations or ranges depending on normality, categorical data as counts and percentages. Differences between groups were evaluated by the student t test or Mann-Whitney U test, depending on the normality of data for continuous data, and by Fisher exact test for categorical data. A two-tailed p-value of less than 0.05 was considered statistically significant. Women were divided into subgroups by the various intervals between last dose of LMWH and delivery (<12 hrs, 12-24 hrs, >24 hrs). Logistic regression was performed for calculating odds ratios for PPH in users and non-users and in relation to timing of last injection of LMWH with adjustment for known risk factors for PPH, such as age, parity and birth weight >4000 gram. Statistical analyses were performed using PASW version 18.0 (IBM SPSS, Chicago, Illinois, United States).

Results

We followed 143 pregnancies in 88 women. We included 88 first pregnancies in which women used full dose anticoagulation. Baseline characteristics are shown in table 1. Median age was 30 yrs (range 20-43 yrs), 66% was nulliparous. All women started with nadroparin. Sixty-eight percent (n= 60) used LMWH during the whole pregnancy, 17 (19%) women switched to acenocoumarol between 16 and 36 weeks, 9 (10%) switched to fondaparinux, 2 (2%) switched to danaparoid and one woman switched to unfractionated heparin during labor. The reason for switching was mostly hypersensitivity skin reactions or (for VKA) the wish to avoid injections. All women used a dosage of anticoagulation intended as therapeutic during pregnancy.

Modes of deliveries were vaginal in 81% and cesarean section (CS) in 19% (9% elective, 10% emergency). Labor was induced in 26 of 88 women: this was in 17/71 (24%) women who had a vaginal delivery, 1/9 (11%) of the women who had an emergency CS and 8 women who had a primary CS. Median gestational age was 39 0/7 weeks (28 3/7-42 3/7). One late fetal loss in the 30th week due to abruptio placenta was reported in this cohort. In total, 3 pregnancies were complicated by preeclampsia and 3 by HELLP syndrome.

Table 1: Baseline characteristics

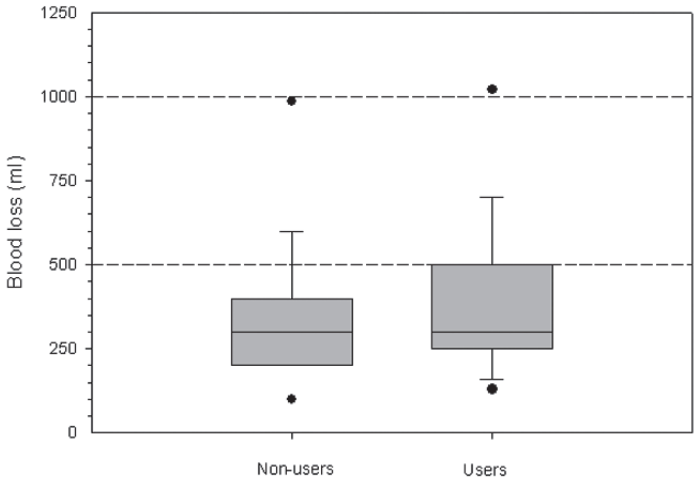
	LMWH users (n=88)	Non-users (n=352)
Median age at pregnancy, years	30	30
Median gestational age at delivery, weeks	39 0/7	39 4/7
Median birthweight, gram	3360	3360
Aspirin use	0	0
Indication for anticoagulation, n(%)		
History of VTE	64 (73)	
Recurrent fetal loss	5 (6)	
Asymptomatic thrombophilic defects[‡]	14 (16)	
VTE in current pregnancy	5 (6)	
Mode of delivery, n		
Vaginal, normal delivery	65	260
Vaginal, assisted	6	24
Cesarean section, primary	8	32
Cesarean section, secondary	9	36

[‡] Antithrombin, protein C and protein S deficiency

Bleeding complications during pregnancy

Of the 88 patients, 5 (6%) had vaginal blood loss during pregnancy without gynecological abnormalities. All these women used LMWH. One patient had epistaxis around the 20th week and 10% had hematoma due to injection of the LMWH. No other bleeding episodes were reported.

Figure 1: Blood loss after vaginal delivery in users vs non-users



Dots indicate 5th and 95th percentiles

Bleeding complications during and after delivery

Risk of PPH after vaginal delivery was 30% vs. 18% for LMWH-users and non-users, respectively (OR 1.9; 95%CI 1.1-3.5; p=0.029). Risk of severe PPH (≥1000 ml) after vaginal delivery was comparable between both groups (5.6 vs 5.0%; OR 1.1; 95%CI 0.4-3.6; p=0.83). Of the LMWH-users who had a severe PPH after vaginal delivery (4/71), 2 had uterine atony, one had retained placenta and one had a PPH after vacuum extraction due to uterine atony. Risk of PPH in women who delivered by CS was 12% (2/17) in LMWH-users and 4% (3/67) in non-users (OR 2.9; 95%CI 0.5-19.4; p=0.26). Both events in LMWH-users occurred after emergency CS, but both had their last injection of LMWH > 12 hours before the CS. See also table 2. The reasons for emergency CS were fetal distress (n=5), prolonged 2nd stage of labor (n=1) and a prolonged 1st stage of labor. (n=3). None of the women with a pregnancy complicated by HELLP-syndrome or pre-eclampsia experienced a severe PPH.

Of the women who used danaparoid (n=2) and unfractionated heparin (n=1) during delivery, none experienced a PPH. Of the women who used fondaparinux during delivery (n=9), one experienced a PPH, but she had her last injection 48 hours before.

The amount of blood loss after vaginal delivery is presented in Figure 1, showing an increased percentage of women with PPH (blood loss 500ml or more), but not of severe PPH (over 1000 ml). Of note, the median blood loss was comparable after primary (350 vs 325ml; p= 0.79) and emergency CS

(425 vs 400 ml; p=0.29) in users vs non-users, respectively.

In total, 8% (2/26) of LMWH users vs 11% (8/73) of the non-users who experienced a PPH, needed red blood cell (RBC) transfusion (p=0.46).

Finally, one woman who used LMWH experienced a secondary PPH (>24 hrs).

Table 2: Risk of PPH in relation to mode of delivery

%	LMWH users (n=88)	Non-users (n=352)	p-value	OR (95%CI)
Overall				
500 ml	29.5	23.6	0.08	1.6 (0.9-2.7)
1000 ml	6.8	4.8	0.46	1.4 (0.5-3.8)
Vaginal delivery (overall)				
500 ml	29.6	17.8	0.029	1.9 (1.1-3.5)
1000 ml	5.6	5.0	0.83	1.1 (0.4-3.6)
Vaginal delivery (spontaneous)				
500 ml	26.1	15.8	0.058	1.9 (1.0-3.6)
1000 ml	4.6	3.8	0.79	1.2 (0.3-4.5)
Vaginal delivery (assisted)				
500 ml	66.7	37.5	0.21	3.3 (0.5-22.0)
1000 ml	16.7	16.6	1.0	1.0 (0.1-11.1)
Cesarean section (overall)				
1000 ml	11.7	4.4	0.26	2.9 (0.5-19.4)
Cesarean section (primary)				
1000 ml	0	6.3	0.45	
Cesarean section (emergency)				
1000 ml	22.2	2.8	0.06	11.3 (1.0-145.5)

Timing of last injection LMWH and risk of bleeding

Of the 88 women with anticoagulation during first pregnancy (LMWH users), 10 women delivered within 12 hours after the last injection of LMWH, 37 women within 12-24 hours and 26 women after 24 hours. In 15 women (17%), the timing was unknown. The time between last injection and delivery of the women who delivered within 12 hours ranged from 5 to 11 hours, with a median of 6 hours. Median blood loss in women who delivered before 12 hours after last dose of LMWH was comparable to 12-24 hours and after 24 hours (275 vs 350 vs 325 ml; p=0.30). Risk of a PPH after vaginal and CS delivery was 30%, 38% and 27% for intervals of <12, 12-24 and >24 hours, respectively (p=0.36). Risk of a severe PPH was 0%, 11% and 4% for intervals of <12, 12-24 and >24 hours, respectively (p=0.58). Overall, the risk of PPH within 24 hours after the last injection of LMWH did not significantly differ from the risk in women who delivered more than 24 hours after the last injection (OR 1.4; 95% CI 0.5-

3.9; p=0.56). After adjustment for age, birthweight >4000 gram and parity, OR was 1.2 (95% CI 0.4-3.6; p=0.73). See for detailed data table 3. In 17% of the women (n=15) we had missing data about the timing of last injection of LMWH, but their median blood loss was comparable with the women who delivered within and after 24 hours (300 vs 350 vs 249 ml; p=0.21).

Of the women who had a planned induction of labor, 50% delivered within 24 hours after the last dose of LMWH and of the women who had spontaneous onset of labor 58% delivered within 24 hours. After adjustment for parity, age and birthweight >4000 gram, LMWH-users who had a spontaneous onset of labor had a 1.9 fold increased risk for PPH compared to women who had a planned induction of labor (95% CI 0.6-5.8; p=0.29).

Table 3: Risk of PPH based on interval between last injection of LMWH and delivery

	< 24 hrs	> 24 hrs	OR (95%CI)	OR (95%CI)‡	*p* ‡
Overall					
MBL, ml	300	350			
PPH, %	34.7	28.0	1.4 (0.5-3.9)	1.2 (0.4-3.6)	0.73
Vaginal delivery					
MBL, ml	300	300			
PPH (≥500 ml), %	35.0	26.3	1.5 (0.5-5.1)	1.3 (0.4-4.8)	0.68
Cesarean section					
MBL, ml	400	400			
PPH (≥1000ml), %	11.1	16.7	0.7 (0.03-12.4)	0.4 (0.1-21.4)	0.67

‡ adjusted for age, parity and birthweight >4000 gram; MBL: median blood loss

Thrombo-embolic complications

No recurrent VTE was reported in the women who used therapeutic dosage LMWH because of a history of VTE. Five women had a current VTE during the pregnancy, one woman had a recurrent VTE in the 8th week and one woman in the 34th week of pregnancy while they had not yet started their thromboprophylaxis. Three patients had a first VTE in 8th, 10th and 37th week of pregnancy and started with therapeutic dosage of LMWH.

Comments

In this study, we analysed the bleeding risk of high dose LMWH during pregnancy and the puerperium in 88 pregnancies. We showed that women who used LMWH had a 1.9-fold increased risk for PPH, but this did not result in more RBC transfusions. We observed no increased risk for a severe PPH after vaginal delivery. The PPH risk was not increased in women who delivered within 24 hours after the last injection of LMWH as compared to women who delivered more than 24 hours after the last dose LMWH.

Overall, we reported 30% PPH (≥500 mL) and 6% severe PPH after vaginal deliveries in women who used LMWH. There are a few studies who also reported the bleeding risk of high dose or therapeutic dosage of LMWH.[7, 10, 13, 16] Kominiarek et al.[7] reported no increased bleeding risk in a case-control study of 49 women using LMWH during pregnancy. Sixty-seven percent used a therapeutic dosage of LMWH, but they did not analyse the risk of therapeutic dosages separately. Furthermore 70% had more than 24 hours between last dose of LMWH and delivery. Voke et al[13] reported a 5% incidence of primary PPH (≥500 mL) in 126 women with a VTE during the current pregnancy followed by therapeutic dosage LMWH, but there was no control-group. Rowan et al[10] described no bleeding complications in 32 pregnancies with therapeutic dosage of LMWH, but 26 of these women had a planned induction of labor. Lepercq et al.[16] described 624 pregnancies of which 49 women used a therapeutic dosage of enoxaparin. They found no increased bleeding risk, but did not analyse the pregnancies with therapeutic dosage separately.

We observed 18% PPH and 5% severe PPH in our matched controls after vaginal delivery. This is comparable with findings of a population-based cohort analysed in the Netherlands, which showed a risk for PPH (≥500 ml) and severe PPH (≥1000 ml) of 19% and 4.2% after vaginal delivery, respectively.[15] There is no single, satisfactory definition for PPH worldwide. In the Netherlands a PPH is defined as ≥1000 ml blood loss for a vaginal delivery and a cesarean section. Because different countries and the WHO define PPH as more than 500 ml blood loss after vaginal delivery, we also used these cut-off values for the analysis. We observed no increased risk for a severe PPH after a vaginal delivery. Moreover, figure 1 shows that the distribution of the amount of blood loss lies mainly below 1000mL. This amount of blood loss is usually not clinical relevant, because this leads mostly not to a RBC transfusion and an extension of the hospital stay.

In our cohort women started with a therapeutic weight-adjusted once daily dosage of LMWH in the beginning of their pregnancy until 37 weeks of gestation. During pregnancy LMWH requirements may alter because the glomerular filtration rate increases in the 2nd trimester and the volume of distribution of LMWH changes. Given these physiologic changes, Crowther et al.[17] suggested that the dosage of LMWH should be increased in proportion to the change in body-weight. However, adjustment of the dose of LMWH according to anti-fXa levels and increasing body-weight is controversial. Some small studies showed that periodic (every 1-3 months) dose-adjustment to maintain therapeutic anti-fXa levels is essential.[18, 19] However, other studies demonstrated that only a few women require dose adjustment when therapeutic doses of LMWH are used.[20,21,22] Therefore, in the absence of large studies with clinical end-points of optimal dosage during pregnancy we decided to not routinely monitor anti-fXa levels and not to adjust the doses for increasing bodyweight or increasing renal clearance.

The women who received their last injection of LMWH within 24 hours before delivery had no increased bleeding risk compared to women who delivered after 24 hours. The ACCP guidelines recommend a weight-adjust twice-daily dosage LMWH and discontinuation of LMWH at least 24 hours prior to elective induction of labor (Grade 1C).[6] In our hospital women who use anticoagulation for (prevention of) VTE have no standard planned induction of labor, but all women switched to divided

therapeutic weight-adjusted dosing of LMWH in the 37th week to minimize the bleeding risk. However, dividing the LMWH dose into twice daily doses and stopping at start of labor may decrease the anticoagulant effect at delivery. In total, only 30% of the women in our cohort had a planned labor. The incidence of PPH in the women who had a spontaneous labor seems to be higher compared to women with a planned labor, (OR 1.9; 95% CI 0.6-5.8; p=0.29) but not reaching statistical significance. This difference cannot be explained by a longer duration between delivery and the last dose of LMWH, because the number of women who delivered within 24 hours was comparable in both groups. Reassuringly, in women who delivered within 12 hours after last dose of LMWH, no severe PPH occurred and no woman needed a RBC transfusion. One study by Maslovitz et al.[8] described the bleeding risk postpartum related to the timing of last injection of LMWH. They found no increased bleeding risk in the women who delivered within 24 hours, but most of these patients (84%) had only a prophylactic dosage of enoxaparin.

Our study has some limitations. Firstly, the amount of blood loss was not objectively measured, but as in common clinical practice the blood loss was a visual estimation. It is known that this gives mostly an underestimation of the amount of blood loss,[23-25] in particular a higher estimated blood loss (>500 ml) is associated with a greater underestimation.[26] In our cohort we observed an increased risk for a ≥500 ml blood loss in vaginal delivery but no increased risk for ≥1000 ml. The latter could be explained by visual underestimation of especially the higher amounts of blood loss. Secondly, the obstetricians attending the birth were not blinded and may have managed the third stage of labor differently in an anticoagulated patient either by administering prophylactic agents (i.e. oxytocics) or intervening earlier to prevent PPH. However, all women (users and non-users) who deliver in our hospital have an active management of the third stage of labor, including oxytocin. Thirdly, we did not evaluate the therapeutic anticoagulant effect with objective laboratory analyses. Therefore, it may be possible that a proportion of patients did not reach therapeutic levels of LMWH in the third trimester. Finally, the collecting of data about the delivery and timing of last dose of LMWH retrospectively is a limitation. Consequently, we had 17% missing data about the timing and two women with missing data had a PPH. However, the median blood loss of these women was comparable with the women without missing data.

Given the increased risk of PPH for vaginal and emergency CS delivery in women using full dose LMWH for prevention of VTE during pregnancy, the individual risk of VTE and PPH should be balanced. We will reconsider our treatment protocol**,** maybe in a subgroup of patients a prophylactic dose of anticoagulation during pregnancy might result in more net benefit.[27] A randomized trial comparing full dose LMWH with prophylactic dose LMWH in pregnant women with an increased risk of VTE is needed to improve patient care.

In conclusion, high dose LMWH carries an increased risk of PPH after vaginal delivery. However, it does not lead to an increased risk for a severe and clinical relevant PPH. It is unknown whether this risk is offset by a lower risk of (recurrent) VTE. Secondly, PPH risk seems to not be increased after deliveries within 24 hours after the last injection of LMWH compared to women who delivered after 24 hours.

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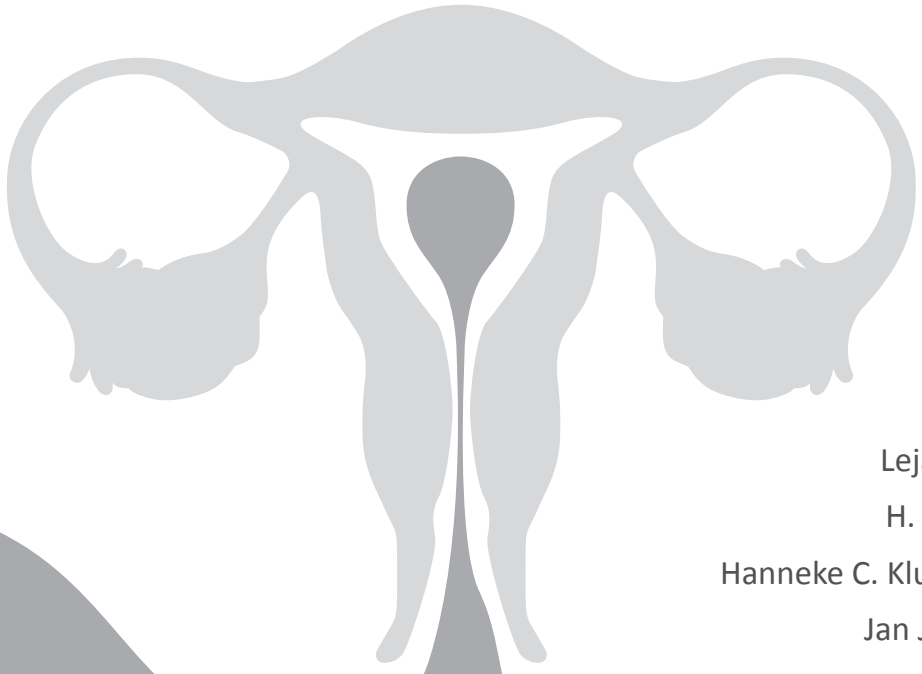
Chapter 6

Incidence of hypersensitivity skin reactions caused by a full dose of low-molecular-weight heparins during pregnancy



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Abstract

Background: Low-molecular-weight heparins (LMWH) are the most commonly used anticoagulants for the treatment and prophylaxis of venous thrombo-embolism in pregnancy. Hypersensitivity skin reactions associated with the use of LMWH are frequently seen, but are probably underreported.

Objective: to evaluate the incidence of hypersensitivity skin reactions due to the use of LMWH in pregnancy, and the subsequent management of anticoagulation.

Patients and methods: from 1999 to 2009, we followed consecutive women who used therapeutic anticoagulation for venous indications. Women visited a combined obstetric/ coagulation clinic and were seen by a thrombosis specialist every two months until six weeks post-partum. All women were started on nadroparin.

Results: we included 135 pregnancies in 88 women. Overall, in 52 of 135 pregnancies (39%), women switched at least once to another anticoagulant due to the development of hypersensitivity skin reactions. Switching to another preparation of LMWH was effective in 77% of the cases. In 23% of the cases skin reactions recurred and another switch had to be made.

Conclusion: in almost half of the pregnancies, women had to switch at least once to another anticoagulant preparation due to the development of hypersensitivity skin reactions on LMWH. In most cases, skin reactions did not recur after switching to a second preparation of LMWH.

Introduction

For pregnant women with either a current venous thrombo-embolism (VTE) or a high risk of recurrent VTE, low molecular weight heparins (LMWH) are the most commonly used anticoagulant. However, hypersensitivity skin reactions are a recognized complication in pregnant patients who use LMWH. Moreover, when this heparin intolerance occurs, alternative choices for anticoagulation are limited and hypersensitivity skin reactions might recur when another preparation of LMWH is used.[1]

Vitamin K antagonist (VKA) could be an alternative anticoagulant, but this drug crosses the placenta and its use in pregnancy is associated with significant fetal risks, particularly teratogenesis and fetal hemorrhage.[2-5] The use of VKA in pregnancy might also be associated with mild neurological dysfunctions in children of school age.[6] Fondaparinux is another alternative anticoagulant treatment, but data on the use in pregnancy are limited.[7]

Rates of mild hypersensitivity skin reactions due to LMWH use in the general population range from 2-7,5%.[8,9] Risk factors for the development of hypersensitivity skin reactions are female sex, obesity and long duration of heparin therapy.[9] It has been hypothesized that the hormonal status may be of influence in the pathogenesis of the delayed hypersensitivity skin reaction to LMWH.[10] Pregnancy also seems to increase the incidence of these skin reactions, ranging from 0.6% to 29%.[1,11-13] These reactions may present as erythematous, well circumscribed lesions without necrosis, usually secondary to a delayed type IV hypersensitivity reactions. An urticarial rash (type I immediate hypersensitivity reaction), skin necrosis and heparin-induced thrombocytopenia have also been reported, although these types of reactions are rare.[10]

A few studies report on the incidence of hypersensitivity skin reactions on LMWH in pregnant women, but these studies had other primary outcomes and therefore hypersensitivity skin reactions are probably underreported.[11-13]

We performed a cohort study in our hospital to assess the safety of the use of a full dose of LMWH in pregnancy. Here, we report the prevalence of hypersensitivity skin reactions of LMWH usage during pregnancy and the subsequent management of anticoagulation.

Patients and methods

Patients

This is a single-centre cohort study, including 88 consecutive women who received a therapeutic dosage of LMWH during pregnancy and the puerperium. All women visited the University Medical Centre Groningen and were followed between 1999 and 2009. We included 135 ongoing pregnancies of these 88 women. Early fetal losses (< 22 weeks of gestation) were not included, due to lack of information on these pregnancies. Indications for anticoagulation were a history of idiopathic, provoked or previous pregnancy related venous thrombo-embolism, a VTE in the current pregnancy, recurrent fetal loss or asymptomatic severe thrombophilic defects (protein C, S or antithrombin deficiency).

Women visited a combined obstetric/ coagulation clinic and were seen by a thrombosis specialist every two months until six weeks post-partum. Information on hypersensitivity skin reactions, episodes of VTE, bleeding, external risk factors for thrombosis, obstetric history, anticoagulant treatment, delivery and pregnancy outcome were collected using a standardised questionnaire and by reviewing medical records. Additional data were added retrospectively. National legislation and the ethical committee of our institution approve this type of studies without the need for review of the protocol.

Treatment protocol

Women were started on a LMWH in early pregnancy, as soon as a pregnancy test was positive or when a VTE occurred. They were all treated with a body weight adjusted therapeutic dosage, during pregnancy and until six weeks post partum. Women with a current VTE during pregnancy were treated for six months, but at least until six weeks post-partum. Women started with a once daily dosage of LMWH, and from the 37th week of pregnancy all women switched to a twice daily dose to minimize the bleeding risk during delivery. Women were instructed about self injection by a research nurse and received an information letter; most women actually injected themselves, but a few were injected by home-care nurses. Anti-fXa levels were not routinely measured and doses of LMWH were not adjusted for increasing bodyweight or increasing renal clearance.

Switch protocol

All women started in the first pregnancy on nadroparin in a weight adjusted therapeutic dosage (175 anti-Xa IU kg^{-1} day^{-1}). When a woman developed hypersensitivity skin reactions, she was switched to tinzaparin in a weight adjusted therapeutic dosage. If the hypersensitivity skin reactions recurred again, the woman was switched to VKA (only during second trimester), dalteparin, danaparoid or fondaparinux. In subsequent pregnancies women started with the preparation that was used without complications during their previous pregnancy.

Definitions

Red pruritic injection infiltrates: itchy, erythematous, well circumscribed lesions without necrosis, subcutaneous, usually secondary to a delayed type IV hypersensitivity reaction.
Generalized rash*:* rash not restricted to the site of injection.
Mild symptoms: symptoms of skin reactions, (including hematomas, pruritic injection infiltrates and non pruritic injection infiltrates) not severe enough to switch treatment (dependent on patient and doctor's preferences)

Statistical analysis

Descriptive statistics were used. The statistical analysis was performed in PASW version 18.0 (IBM SPSS, Chicago, Illinois, United States).

Results

Eighty-eight women had 135 pregnancies between 1999 and 2009. Twelve of these women (=12 pregnancies) were also included in a study of Bank et al.[1] Median maternal age was 30 years (range 20-43). Indications for anticoagulation were previous VTE in 98 (73%) pregnancies, a current VTE in 5 (4%) pregnancies, an asymptomatic thrombophilic defect in 23 (17%) pregnancies and recurrent fetal loss in 5 (4%) pregnancies. In four (3%) pregnancies therapeutic dosage of LMWH was given for other reasons (strong positive family history for VTE). In 66 (49%) of the pregnancies women were nulliparous. None of the patients had a history of thrombocytopenia or an allergy to a LMWH. Baseline characteristics are displayed in table 1.

Table 1: Baseline characteristics of study population

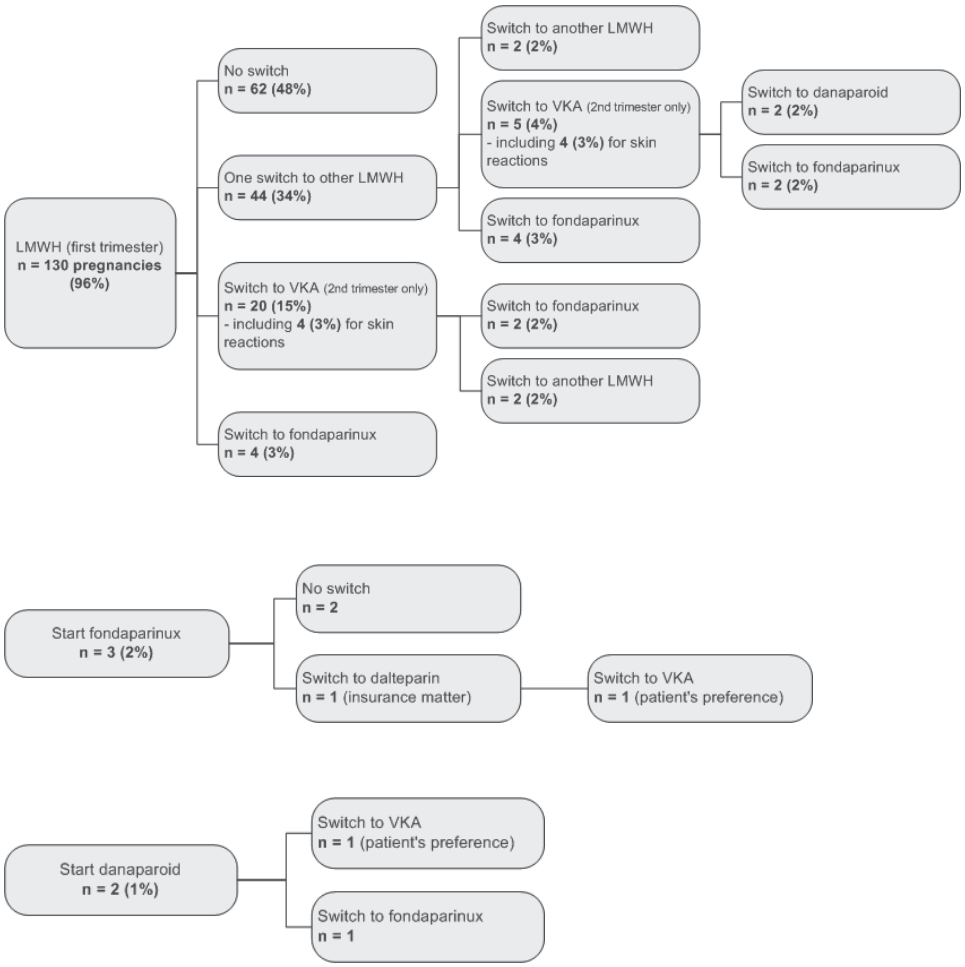
Women, n	88
Pregnancies, n	135
Maternal age (median, range)	30 (20-43)
Parity	
Nulli-	66 (49)
Multi-	69 (51)
Gestational age at delivery in weeks (median, range)	39.4 (27.5-42.3)
Birthweight in gram (median, range)	3372 (750-4890)
Indication for anticoagulation during pregnancy	
VTE in current pregnancy, n (%)	5 (4)
Previous VTE, n (%)	98 (73)
Asymptomatic thrombophilia, n (%)	23 (17)
Recurrent fetal loss, n (%)	5 (4)
Other (strong positive family history for VTE)	4 (3)
Pregnancy outcome	
Liveborn	129
Congenital birth defects	4
Stillborn	1
Late fetal loss (>22 weeks)	3
Termination due to severe fetal anomalies	2

Pregnancy outcomes

Hundred-and-thirty-five pregnancies, including one twin pregnancy, resulted in 129 live infants. Median gestational age of live infants was 39.4 weeks, ranging from 27.5 to 42.3 weeks. Median birth weight was 3372 gram, ranging from 750 to 4890 gram. Three late fetal losses (23-27 weeks) were observed. In one of these three pregnancies VKA was used during the second trimester. In addition,

one infant was stillborn due to placental abruption at 31 weeks and two pregnancies were terminated, for severe fetal anomalies (trisomy 18 and severe cardiac defect). Four live born infants had congenital defects: a cleft palate, clubfeet and a (genetic form of) retinoblastoma: no VKA were used in these pregnancies. One male infant was born with an epispadia, in this pregnancy VKA were used during the second trimester. Results are also displayed in table 1.

Figure 1: Switches of anticoagulation treatment during pregnancy



Used anticoagulation

Overall, in 52 out of 135 pregnancies (39%), women switched at least once to another treatment due to the development of hypersensitivity skin reactions. In 44 pregnancies (34%) women switched

to another LMWH, thereafter in 77 % (n=34) no other switch in treatment was required. In two pregnancies (2%) women switched twice to a different LMWH and in eight pregnancies (6%) women switched to VKA in the second trimester, due to the recurrence of hypersensitivity skin reactions. In 19 pregnancies (14%) women switched to VKA for other reasons, such as aversion to injections or patients' preferences. In sixty-two pregnancies (46%) women continued using LMWH during their whole pregnancy and puerperium without hypersensitivity skin reactions, or with only mild reactions not severe enough to switch. (see also figure 1)

Four women used danaparoid, one of whom developed a generalized rash, while one woman developed mild symptoms but continued using danaparoid.

Fondaparinux was used in fifteen pregnancies (11%) because of hypersensitivity skin reactions to at least one type of LMWH in the current or previous pregnancy. No skin reactions were observed with the use of fondaparinux. These results were described elsewhere.[7]

Taking into account only the first pregnancies (n=88), all women started on nadroparin. Overall, in 37 (42%) first pregnancies, women were switched at least once to another anticoagulation treatment for hypersensitivity skin reactions. In the subsequent pregnancies, this incidence was lower: in only 13 pregnancies (28%) women were switched to another anticoagulation treatment for hypersensitivity skin reactions.

Type of skin reactions

LMWH were used in 131 pregnancies. Pruritic erythematous infiltrates on the site of injection due to the use of LMWH were observed in 38% (n=50) of pregnancies. Mild symptoms, which did not require a switch in treatment occurred in another 25% (n=33) of the pregnancies. Results are displayed in table 2.

Table 2: Reported side effects of pregnancies on LMWH

	LMWH-users (n= 131)
No side effects, n(%)	46 (35)
Pruritic injection infiltrates, n(%)	50 (38)
Rash (generalized), n(%)	2 (2)
Mild symptoms, n (%)	33 (25)

A more generalized rash was observed in 2% (n=2) of the pregnancies. One woman developed a rash on the sites of injection, in the groin, armpits, elbows and knees on nadroparin. No complications were observed with the subsequent use of dalteparin. The second woman developed erythematous pruritic injection infiltrates on both thighs, which expanded during the use of nadroparin and dalteparin. The woman was switched to danaparoid, but developed red itchy expanding infiltrates

again. Finally, the delivery was initiated and post partum she received a vitamin K antagonist. In the next pregnancy she was treated with compression stockings during the first trimester, VKA during the second trimester and fondaparinux during the third trimester without complications.

Discussion

In this study, we evaluated the use of a therapeutic dosage of LMWH during pregnancy. Overall, in 39% of the pregnancies, women had to switch at least once to another LMWH, acenocoumarol, danaparoid or fondaparinux for the development of hypersensitivity skin reactions. In the first pregnancies with a full dose anticoagulation (n=88) the rate was even higher; 42% switched at least once to another anticoagulation treatment. Switching to another preparation of LMWH seems to have a good effect, because in 77% of these pregnancies no second switch was needed.

Compared to other studies our rate exceeds the highest reported rate of 29% by Bank et al.[1] They reported a prospective, observational study, including 66 pregnant women. They found a skin complication rate of 29%, these skin complications consisted of itching (20%), local redness (23%), subcutaneous infiltrates at the injection site (11%), pain during injection (3%) and a generalized rash (3%). To maintain a consecutive cohort, data of 12 pregnancies included in the study of Bank et al[1] were also included in our study, but excluding these pregnancies did not change our results. Other studies that assessed the usage of LMWH during pregnancy had mostly bleeding or thrombotic complications as a primary outcome. Two reviews evaluated the complication rate of LMWH and reported also skin reactions as a secondary outcome. First, Sanson et al[12] performed a review of 21 studies, including 486 pregnancies. They found only three cases (0.6%) of diffuse skin reactions, which led to cessation or change of treatment. Second, in a review by Greer and Nelson-Piercy,[11] 64 reports were included with in total 2777 pregnancies. They found that 1.8% of women using LMWH in pregnancy developed allergic skin reactions. We think that the high rate of skin complications we report here is real. Wütschert et al[10] also suggested in a review that the incidence of hypersensitivity skin reactions on LMWH might be underreported. In our hospital women were followed with a focus on adverse events, which may be an explanation for the higher incidence of reported hypersensitivity skin reactions. However, the true percentage of hypersensitivity skin reactions in this cohort seems to be even higher, because some women did develop mild skin reactions, but did not switch to another treatment.

Different types of hypersensitivity skin reactions are described.[10] Most common is the delayed type IV hypersensitivity reaction, other reactions include type I immediate hypersensitivity reactions, skin necrosis and heparin-induced thrombocytopenia.[10] In our study the delayed type IV reaction was also most commonly observed.

Fondaparinux was used in 15 pregnancies in our study. No hypersensitivity skin reactions were observed, but the use of this drug is limited by the fact that it crosses the placenta.[14] The use of fondaparinux in this study population was already described elsewhere.[7]

Schindewolf et al[9] described an increased risk for developing hypersensitivity skin reactions for a

body mass index greater than 25, duration of heparin therapy longer than nine days and female sex. They reported an overall incidence of 7.5%, but they included only a few pregnant women. However, our cohort of women had at least two of these risk factors. Unfortunately, we had no information about the BMI, so we could not analyze this relation.

A limitation of our study was the clinical diagnosis of the skin reactions. The interpretation and the decision to switch to another anticoagulant was based on a clinical diagnosis made by different doctors, and was not objectified by skin tests. On the other hand, there is also no consensus in the literature about how to test skin allergy to LMWH.[10] Wütschert et al[10] recommended switching to another LMWH without prior skin tests. Another review by Bircher et al,[15] concluded that in some anticoagulant-associated hypersensitivity reactions detailed allergologic investigation may help to identify safe treatment alternatives. However, several tests may be needed, and the procedures are usually time-consuming. Although the interpretation of the hypersensitivity skin reactions was based on a clinical diagnosis in our cohort, symptoms were troublesome enough to change therapy.

Because of the study design, we cannot compare the different preparations of LMWH. There is a bias by indication; all women were started on nadroparin and other preparations were only used when a woman already had shown hypersensitivity skin reactions to nadroparin. Switching treatment seems to have a good effect, but we cannot exclude that longer duration of exposure to LMWH might also decrease the development of hypersensitivity skin reactions.

Our findings should lead to an altered view of hypersensitivity skin reactions during pregnancy. Physicians should be aware that patients receiving LMWH have a high risk of developing a delayed type IV hypersensitivity reaction. Therefore, we recommend monitoring pregnancies with anticoagulant treatment to recognize skin reactions. This might help patients to adhere to anticoagulant therapy.

In conclusion, we report here a rate of 39% hypersensitivity skin reactions in women on LMWH during pregnancy. These reactions can primarily be managed by changing therapy to another preparation of LMWH, which is successful in 77% of the patients. In a subgroup of women, it is necessary to ultimately switch to VKA or fondaparinux.

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Chapter 7

Fondaparinux as an alternative anticoagulant therapy during pregnancy

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Abstract

Low-molecular-weight heparins (LMWHs) are widely used in pregnant women in whom anticoagulant therapy is indicated, but they frequently cause hypersensitivity skin reactions. Choices of alternative anticoagulation are limited. In our hospital, experience with fondaparinux as an alternative in pregnant patients has accumulated. This report describes ten patients who used fondaparinux during 12 pregnancies between 2003 and the present. All women were followed prospectively. They initially used LMWH but developed hypersensitivity skin reactions. Two patients used fondaparinux during two pregnancies, both started in the 1st trimester of the 2nd pregnancy. In all other pregnancies, fondaparinux was started in the 2nd or 3rd trimester. Fondaparinux was not associated with skin reactions or other side-effects. None of the 13 infants had congenital abnormalities or neonatal bleeding. We report here an alternative anticoagulant treatment with fondaparinux in 12 pregnancies who had allergic skin reactions to LMWH.

Hypersensitivity skin reactions are frequently seen in pregnant patients who use low-molecular-weight heparin (LMWH).[1] When this heparin intolerance occurs, alternative choices for anticoagulation are limited. Hypersensitivity skin reactions often recur when another preparation of LMWH is substituted.[1] Danaparoid is another preparation of choice which does not pass the placenta,[2] but it is not consistently available. Most patients strongly wish to avoid vitamin K antagonists, even beyond the twelfth week of pregnancy, because of the association with congenital and developmental abnormalities.[3,4] Fondaparinux, a synthetic selective inhibitor of activated factor X (fXa), is commonly used as an alternative anticoagulant in non-pregnant patients who develop heparin intolerance. Fondaparinux has been extensively studied for use in surgery prophylaxis and treatment of thromboembolic diseases.[5] However, data on the use of fondaparinux in pregnancy is limited to animal models and a few case reports.[6-10] Although it has been shown that transplacental passage can occur resulting in low measurable fXa activity in cord blood,[11] no adverse outcomes in pregnancy have been reported in these four women. The other six separately published cases reported no adverse events to mother and child either.[6-10] In our hospital, experience with fondaparinux as an alternative in pregnant patients has accumulated. The aim of our study was to evaluate the use and safety of fondaparinux during pregnancy.

From 2003 until the present, we prospectively followed a consecutive cohort of 133 women in our university hospital who used anticoagulant therapy during their pregnancy and puerperium. The indication for anticoagulant therapy was a history of idiopathic, provoked or previous pregnancy-related venous thrombo-embolism (VTE) or recurrent fetal loss. Recurrent fetal loss was defined as 2 or more fetal losses. In all women thrombophilia screening was performed. Applied assays have been described elsewhere.[12] All women were started on a LMWH (nadroparin or tinzaparin) in early pregnancy with body weight adjusted therapeutic dosage (175 anti-Xa IU kg^{-1} day^{-1}), as soon as a pregnancy test was positive. All women were followed in a combined obstetric/ coagulation clinic and seen by a thrombosis specialist every 2 months until 6 weeks post-partum. Anti-fXa levels were not routinely monitored in maternal and cord blood and doses of LMWH were not adjusted for increasing bodyweight or increasing renal clearance. If hypersensitivity skin reactions developed, we either switched once to another preparation (tinzaparin or nadroparin), acenocoumarol or started fondaparinux 2.5 mg subcutaneously twice daily. Fondaparinux 7.5 mg once daily (therapeutic dosage) is not available in the Netherlands, and we wished to avoid thrice daily injections. The women had no standard planned induction with withholding of fondaparinux, but anticoagulation was stopped at start of spontaneous labour and restarted 4-8 hours after delivery (when blood loss was normal) and stopped six weeks post partum. Data were systematically collected by the thrombosis physicians during pregnancy on indication for anticoagulant therapy; start date of LMWH; trimester of switching to another LMWH, vitamin K antagonist or fondaparinux; bleeding and thrombo-embolic complications during pregnancy, delivery and post-partum period; side-effects of anticoagulants; gestational age of delivery; blood loss during delivery; birth-weight and neonatal bleeding/congenital abnormalities. National legislation and the ethical committee of our institution approve this type of studies without

the need for review of the protocol. All women were informed about the risks of the off label use of fondaparinux during pregnancy and agreed with it. In this letter, we report on the 12 out of 190 pregnancies in 133 women in which fondaparinux was used.

We treated 10 patients with fondaparinux during pregnancy and the puerperium in our institution, two of them during two pregnancies. Their median age was 30 yrs (range 26-34 yrs). Six of them had a history of VTE during combined oral contraceptive use, three patients had a history of an idiopathic VTE and one patient had a history of recurrent fetal loss. None of the patients had a history of allergy to LMWH. Eight patients were switched to fondaparinux in the 2nd trimester and 2 patients during the 3rd trimester. All patients were started initially on nadroparin or tinzaparin, two used both preparations and one patient used a vitamin K antagonist during the 2nd trimester before switching to fondaparinux. See for detailed information Table 1. In all cases, the indication for switching to fondaparinux was hypersensitivity skin reactions to LMWHs, consisting of itching, local redness or subcutaneous infiltrates localized at the injection sites. Two patients were treated during two pregnancies, both started fondaparinux in the first trimester of their second pregnancy. No hypersensitivity skin reactions to fondaparinux were seen and patients reported no other side effects. No early or late fetal losses occurred. The median gestational age at delivery was 39 weeks (range 33 5/7-42 0/7 weeks). One patient delivered preterm twins due to preterm prelabour rupture of the membranes. The median blood loss during delivery was 450 ml (range 200-2000 ml), three patients had more than 1000 ml blood loss. The patient with 2000 ml blood loss had an atony of the uterus, she received her last injection of fondaparinux more than 12 hours before delivery. One patient had 1200 ml blood loss during a secondary section caesarean, she received her last injection 48 hours before delivery. The third patient had 1000 ml blood loss due to atony of the uterus and a preterm delivery of a twin, she received her last injection 7 hours before delivery. None of these women with more than 1000 ml blood loss needed a blood transfusion. Postpartum, 4-8 hours after cessation of the bleeding, these patients restarted on fondaparinux 2.5 mg twice daily. No bleeding recurred after restarting the drug. None of the 13 infants had congenital abnormalities or neonatal bleeding. Their median birth-weight was 3685 gram (range 1795-4330 g). No minor or major bleedings or thromboembolic events were reported during pregnancy or post-partum period.

We report on all 12 pregnancies in our centre in which fondaparinux was used during the past six years. These data are derived from a prospective cohort study. We show that fondaparinux was not associated with increased bleeding, thromboembolic complications or fetal abnormalities.

To our knowledge, this is the largest prospective study that reports on the use of fondaparinux during pregnancy. In concurrence with others,[6-11] we did not observe hypersensitivity skin reactions on fondaparinux, although all women had had hypersensitivity skin reactions to LMWHs. Recurrence of hypersensitivity skin reactions when switching to another preparation of LMWH in pregnant women is a known phenomenon.[1]

Fetal safety is an important issue when considering a new anticoagulant therapy in pregnancy. LMWHs do not cross the placenta, so cannot cause teratogenicity or neonatal bleeding.[13-15] A study by

Dempfle et al. demonstrated that fondaparinux passes the placental barrier in vivo, resulting in low measurable anti-fXa levels in umbilical cord blood.[11] That study described five pregnant women, in four of whom low anti-fXa levels were measured. These levels were approximately 1/10 the concentration in maternal plasma, which is well below the concentration required for effective anticoagulation.[16] Neonatal bleeding did not occur in the infants of these women. In the fifth woman, no elevated anti-fXa level was measured, probably because she received her last injection of fondaparinux 22 hours before delivery. Based on these data, fondaparinux has the potential to affect the fetus. In a recently published retrospective study by Winger et al.,[17] 29 women with a history of unexplained recurrent fetal loss and infertility using fondaparinux 2.5 mg once daily during the 1st trimester of pregnancy were described. They reported no adverse events, in particular no fetal abnormalities. In the 13 infants in our study, no congenital abnormalities or bleeding occurred. Our data are obviously limited by the size of our study, although it is the largest prospective series reported. Of note, only 2 infants were exposed during the first trimester. A limitation of our study is the missing of anti-fXa levels in maternal and cord blood during labour because of the possibility of passage through the placenta and the potential side-effects for mother and child. Nevertheless, none of the children had neonatal bleeding or fetal abnormalities.

In conclusion, we report here an alternative treatment with fondaparinux in 12 pregnancies in 10 women who had hypersensitivity skin reactions to LMWH. Fondaparinux did not cause hypersensitivity skin reactions and was not associated with bleeding or other complications in mother and child. However, given the limited data, the use of fondaparinux during the first trimester should still be avoided.

Table 1: Detailed patient characteristics

Case no.	Age	Indication for anticoagulation	Thrombophilia	Anticoagulation before fondaparinux	Trimester start fondaparinux	Gestational age at delivery	Birth weight (gram)	Blood loss during delivery (ml)	Last injection before delivery (hrs)
1	32	PE during COC	PS def type I / hetzg FV leiden	nadroparin	3rd	40 4/7	3670	200	25
1	34	PE during COC	PS def. type I /hetzg FV leiden	-	1st	40 4/7	4330	300	20
2	30	Idiopathic DVT	Hetzg FV leiden	tinzaparin	2nd	38 6/7	3450	700	> 12
3	30	recurrent fetal loss	No	nadroparin	2nd	38 2/7	3570	1200 †	48
3	32	recurrent fetal loss	No	-	1st	39 0/7	3975	500	17
4	30	CVT during COC	No	nadroparin/ VKA	2nd	42 0/7	3929	2000 ‡	> 12
5	30	DVT during COC	No	nadroparin/ tinzaparin	2nd	37 0/7	3070	400	22
6	32	Idiopathic DVT	No	nadroparin	2nd	40 3/7	3700	300	7
7	26	Idiopathic PE	No	nadroparin	2nd	33 5/7	1990/ 1795	1000 ‡	7
8	28	DVT during COC	PS def.type III / hetzg FV leiden	nadroparin	2nd	37 6/7	3110	200	9
9	33	PE during COC	PS def type I	nadroparin	3rd	41 1/7	3795	300	31
10	29	DVT during COC	No	nadroparin/ tinzaparin	2nd	42 0/7	3795	700	30

CVT= cerebral venous thrombosis, DVT= deep venous thrombosis, PE= pulmonary embolism, VKA=vitamin K antagonist, COC= combined oral contraceptive use, PS= protein S, def=deficiency, FV= factor V, hetzg=heterozygous. ‡ secondary section caesarean † atony of uterus

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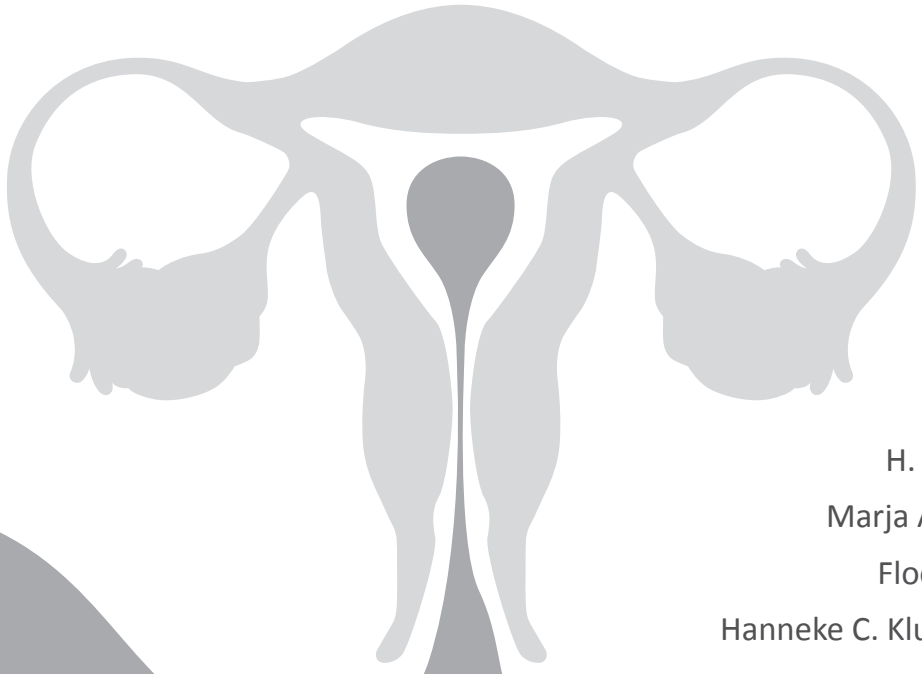
Chapter 8

Reproductive choices and obstetrical experience in Dutch carriers of haemophilia A and B



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Abstract

Introduction: reproductive choices, pregnancy and childbirth are influenced by culture and traditions. This probably also plays a role in carriers of haemophilia.

Aim: to evaluate the reproductive choices and obstetrical experiences in the current generation of carriers of haemophilia in our Haemophilia Centre in the north of the Netherlands, a largely secular country with liberal abortion laws and a unique tradition of home births.

Methods: retrospective survey among haemophilia carriers.

Results: we sent a questionnaire to 74 carriers, 65 were available, 75% responded. Median age was 41 (range 20-83) years. 46/49 women had 120 pregnancies: 25 resulted in fetal loss, two in pregnancy termination (one for haemophilia) and 93 in live births. No woman had chosen not to start a family. Mean number of children was 2.0, 2.4 vs 1.8 in women with and without sons with haemophilia (p=0.008), respectively. Twenty women (20/46) were unaware of their carriership during 1st pregnancy, they were younger at 1st pregnancy than known carriers (25 vs 29 years, p=0.03). Twenty-three percent reported bleeding complications during the first delivery. Overall, 10% versus 3% of deliveries was complicated by a primary and secondary postpartum haemorrhage (PPH), respectively.

Conclusion: in our Haemophilia Centre, carrier state has not influenced reproductive choices in the past, other than older age at first pregnancy. Carriers of haemophilia have an increased risk of primary PPH.

Introduction

Culture and customs play an important role in reproductive choices, pregnancy and childbirth. This is probably also true in carriers of haemophilia. Until not very long ago, carriers of haemophilia hardly had options regarding reproduction other than accepting the birth of affected sons. In the Netherlands, the widespread use of contraceptives started in 1960 when the 'pill' became available. Of the women aged 16-49 years 40-50% used oral contraceptives between 1990 and 1999 in the Netherlands.[1] Of the women aged 20-29 years even 69% used oral contraceptives.[1] More than 90% percent of pregnancies in the general population are planned.[1] Testing for carriership of haemophilia and consequently prenatal diagnosis by chorionic villous biopsy became available in the mid 1980s.[2,3] Elective pregnancy termination for social and medical reasons has been widely accessible since 1984. More recently, advances in molecular science and ultrasound technology have provided more prenatal diagnostic options for carriers of haemophilia. Preimplantation genetic diagnosis for haemophilia has been available to our patients since 1995. Preimplantation genetic testing (PGD) is an alternative to conventional prenatal diagnostic techniques (amniocentesis, chorionic villus sampling) that allows couples to avoid intrauterine transfer of male embryos and consequently the possibility of an affected embryo. It is an adjunct to assisted reproductive technology, and requires in vitro fertilization to obtain oocytes or embryos for evaluation.

In the Dutch obstetrical care system, women with low risk pregnancies can choose between home and out-patient hospital delivery. About 30% of the deliveries take place at home.[4] This means that in the past, and also presently in women with unknown carrier state, women with unknown levels of plasma factor could be at risk of bleeding during pregnancy and delivery in a low-tech environment. From 2000 on, national guidelines advise to refer all known carriers, irrespective of factor levels and fetal sex, to the obstetric department of a hospital with a Haemophilia Centre. Despite advances in care, pregnancy and delivery remain critical times for carriers of haemophilia, with previous studies reporting 9-38% bleeding complications.[5-8] The aim of this study was to evaluate the reproductive choices and obstetrical experiences in the current generation of carriers of haemophilia in our Haemophilia Centre in the north of the Netherlands.

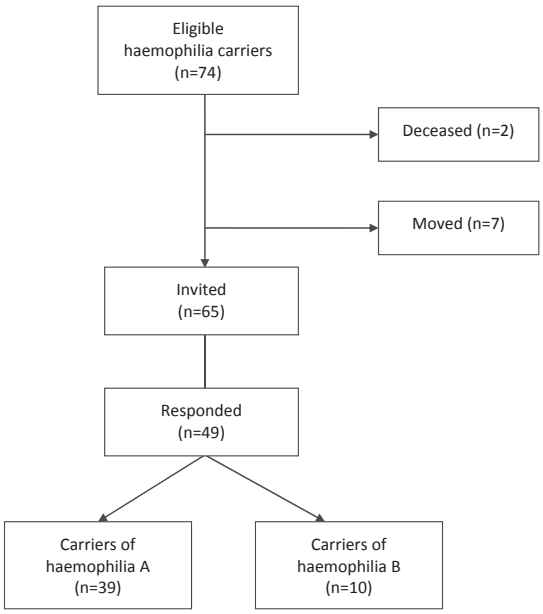
Material and methods

We identified all carriers of haemophilia A and B registered in our Haemophilia Centre in 2007. A standardised questionnaire was sent to all carriers above 18 years of age. The questionnaire contained questions about their experience of pregnancies, deliveries and reproductive choices. The questionnaire was split into three sections describing: carrier state, complications during pregnancy and delivery and influences of reproductive choices. We recorded data about pregnancies, number of fetal losses and termination of pregnancy, place of delivery, complications during delivery; knowledge of carrier state during 1st pregnancy and experiences that influenced their reproductive choices. The questionnaire included yes/no, multiple choice and open-ended questions. Clinical details including

type (A or B), severity of haemophilia in the family and non-pregnant factor VIII and IX levels were obtained from medical records. National legislation and the ethical committee of our institution approve this type of retrospective study without the need for review of the protocol. Primary and secondary postpartum haemorrhage (PPH) was defined as a bleeding within 24 hours after delivery and after 24 hours, respectively.[9,10] We classified a family as complete if the youngest child was older than five years.

We analysed the data with Statistical Package for the Social Sciences (SPSS) version 16.0. Descriptive statistics were used; results were compared between carriers of a known and unknown carrier state during 1st pregnancy. Differences between groups were evaluated by the student t test or the Mann-Whitney U test; carriers who have never been pregnant were excluded from this analysis.

Figure 1: Flow diagram cohort



Results

We sent the questionnaire to 74 carriers. Two women were deceased, seven had moved and the other carriers did not respond. Of 65 women, 49 (75%) women responded. See also Figure 1. The median age was 41 (range 20-83) years. All carriers were Caucasian. Eighty percent were carrier of haemophilia A and 20% were carrier of haemophilia B. Twenty-three, 11 and 15 carriers had a family with severe (<1%), moderate (1-5%) and mild (>5%) haemophilia, respectively. The median non-pregnant value of factor VIII and IX was 0.52 IU/ml (range 0.17-1.44) and 0.49 IU/ml (range 0.16-0.87 IU/ml), respectively. See also table 1.

Table 1: Characteristics

	Carriers (n=49)
Median age (range)	41 (20-83)
Median number of pregnancies (range)	2 (0-9)
Mean number of live births (SD)	2.0 (1.0)
Carrier state (n (%))	
Haemophilia A	39 (80)
Haemophila B	10 (20)
Carrier state known at 1st pregnancy	26 (57)
Median non-pregnant factor level (IU/ml)	
Factor VIII (range)	0.52 (0.17-1.44)
Factor IX (range)	0.49 (0.16-0.87)
Pregnancy outcome (n)	
Pregnancies	120
Live births	93
Fetal losses	25
Termination of pregnancy	2

Pregnancy outcomes

Forty-six carriers had 120 pregnancies between January 1956 and November 2007. Twenty-five resulted in fetal loss, two in terminations of pregnancy and 93 in live births. One carrier delivered a twin. Three of the carriers had never been pregnant, because they had not yet a child-wish during survey (aged 23, 26 and 36). Three women (7%) experienced two or more fetal losses. Two women had a pregnancy termination, one for haemophilia. Forty-six women (94%) had been pregnant at least once, 43 of them had at least one ongoing pregnancy. Of the carriers 32 had a completed family during survey. Of the infants born 59% were male, 53% of them were confirmed to have haemophilia. Of the 43 women 63% had one or more affected sons. The mean number of children was 2.0 (SD 1.0), 2.4 vs 1.8 in women with and without sons with haemophilia (p=0.008), respectively.

Reproductive choices

Twenty women (20/46) were unaware of being a carrier of haemophilia during their 1st pregnancy. Carriers with an unknown state were younger at their 1st pregnancy compared to carriers with a known state (25 vs 29 years, p=0.03). The number of pregnancies and live births were comparable in known and unknown carriers. See also Table 2. The mean number of live births were comparable in carriers with a family history of severe, moderate and mild haemophilia. (2.0 vs 2.5 vs 1.9, p=0.25) Two known carriers decided to termination of one pregnancy, one for fear of passing haemophilia on to a child and one for social reasons. None of the carriers had made the conscious choice to have no children.

Five women chose to have no more children, four because of the concern for an affected son and one because of the bleeding risk during delivery. Two women in a family with severe haemophilia used preimplantation genetic diagnosis for gender selection before pregnancy, leading to the birth of twins in one woman.

Table 2: Comparison known and unknown carriers at 1st pregnancy

	Known carrier state (n=26)	Unknown carrier state (n=20)	p-value
Mean age at 1st live birth (yrs)	29	25	0.03
Median number of pregnancies	2	3	0.23
Mean number of live births	1.8	2.3	0.13
Termination of pregnancy (n)	2	0	
Delivery location, n			
At home	6	17	
General hospital	16	25	
Haemophilia centre	23	6	
Complications, n			
Bleeding after fetal loss	0	0	
Postpartum bleeding	7	7	
primary	4	5	
secondary	1	2	
unknown	2	-	
Mode of delivery, n			
Vaginal	44	41	
Section caesarean	4	4	

Delivery and complications

None of the women who experienced one or more fetal losses reported bleeding complications. The modes of delivery were vaginal delivery in 85 (91%) and caesarean section in 8 (9%) pregnancies (2 elective and 6 emergency). The mode of delivery was comparable between the carriers with a known and unknown state. (See for detailed information Table 2). Births took place at home in 23 cases, in a general hospital in 41 cases and in a Haemophilia Centre in 29 cases, respectively. The deliveries at home took place between 1956 and 2000. Eleven of the 43 women (26%) reported bleeding complications during one or more deliveries. Overall, 15% (14/93) of the deliveries was complicated by a primary or secondary PPH. Twenty-three percent (10/43) reported bleeding complications during or after the first delivery. Nine of them had a vaginal delivery and one a secondary section caesarean. Six of them had a primary PPH. None of these women received prophylactic factor VIII or IX. Bleeding

complications during the 2nd delivery was reported by 12% (4/33), 3 of them had a primary PPH, all delivered vaginally. One woman received postpartum administration of factor VIII concentrate. None of the 17 women reported bleeding complications during the 3rd and 4th delivery. Overall, 10% versus 3% of the deliveries was complicated by a primary and secondary PPH, respectively. The timing of two PPHs was unknown. Of the carriers who delivered at home, in a general hospital and in a Haemophilia Centre, 4% versus 21% versus 10% experienced a PPH. Of our cohort, 16 carriers had non-pregnant factor VIII or IX levels below 0.50 IU/ml. The incidence of PPH was comparable between the carriers with non-pregnant levels below 0.50 IU/ml and carriers with a non-pregnant factor level above 0.50 IU/ml. The median non-pregnant factor levels of the carriers with a history of PPH and no bleeding complications were comparable, 0.56 versus 0.52 IU/ml, respectively (p=0.82).

Discussion

In the present study we evaluated the reproductive choices and obstetrical experiences of carriers of haemophilia A and B. We reported that in our Haemophilia Centre, the knowledge of carrier state has not influenced reproductive choices in the past except for older age at first pregnancy. Known and unknown carriers had the same number of children. The self-reported risk of PPH was increased in carriers of haemophilia.

The mean number of live births in our cohort was 2.0 and the mean number of live births was comparable between known and unknown carriers (1.8 vs 2.3). The mean number of live births per woman is comparable with the general Dutch population in which the mean number of live births is 1.8 per woman.[11] Despite the social acceptance and legalization of provoked abortion in the Netherlands, only one woman in our cohort had decided to termination of one pregnancy because of the concern of an affected son. The mean number of children in women with an affected son was significantly higher compared to women without an affected son. (2.4 vs 1.8, p=0.008). Having a son with haemophilia was apparently not a reason to have no more children. On the other hand, known carriers were older at the 1st pregnancy compared to unknown carriers, which is in line with a Swedish study about reproductive choices in carriers of haemophilia.[12]

Bleeding complications after one or more deliveries were reported by 26% of the women in our cohort. Another assessment by questionnaire of PPH in carriers reported also an incidence of 22%.[13] We demonstrated an increased incidence of primary PPH (10%). This is comparable with other studies of carriers of haemophilia who reported also an increased risk of bleeding complications primary post-partum.[5,6] In the general Dutch population the incidence of a primary PPH (>1000ml) during the first delivery is 4%.[9] The incidence of a secondary postpartum haemorrhage in the general Dutch population is 2%, this is comparable with our cohort.[14] The tendency to bleed in carriers of haemophilia could be explained by low plasma levels of factor VIII and IX. In our cohort there is no difference between the median non-pregnant factor levels of the carriers with a PPH compared to the women without bleeding complications, 0.56 versus 0.52 IU/ml, respectively. The incidence of PPH in

carriers of haemophilia is higher in a general hospital compared to a haemophilia centre (21 vs 10%).

This study had some limitations. Firstly, in the self-reported questionnaire, bleeding symptoms might have been overreported as well as underreported. In some cases, oozing might have been reported as bleeding. On the other hand, in families where blood loss is a well-known and accepted phenomenon, abnormal bleeding might not have been recognized as such. The response rate in our study was 75%, therefore the results may not represent an overview of experiences of all carriers. Probably, the response rate of carriers with negative experiences is higher compared to women with no complications during pregnancy and delivery. Secondly, the inclusion of older women limits its applicability to future patients when we consider more advanced prenatal diagnostic options like chorionic villous biopsy and PGD. However, most options were already available to the majority of known carriers in our cohort.

In conclusion, in our Haemophilia Centre carrier state has not influenced reproductive choices in the past, other than older age at first pregnancy. We confirm that carriers of haemophilia have an increased risk of primary PPH, but not of secondary PPH.

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Chapter 9

High thrombin activatable fibrinolysis inhibitor (TAFI) levels may protect against recurrent fetal loss

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Abstract

Fetal loss may be induced by apoptosis of trophoblasts due to high levels of fibrin degradation products. Consequently, high TAFI levels would reduce this risk, especially in women with thrombophilia who generate more thrombin. This hypothesis was tested in women from 4 family cohorts, originally designed to estimate the risk of venous thromboembolism, associated with either hereditary deficiencies of antithrombin, protein C or protein S, prothrombin 20210A, factor V Leiden, high factor VIII levels or hyperhomocysteinemia. We retrospectively assessed fetal loss rates in women with high TAFI levels ($\geq 75^{th}$ percentile, i.e. 115 U/dl), compared to women with normal TAFI levels. Of 1557 women, 843 (probands and relatives) were evaluable of whom 213 had high TAFI levels. Women with high TAFI levels and women with normal TAFI levels were comparable for age at time of first pregnancy (24 vs 24 years) and mean number of pregnancies (2.9 vs 3.0). Thrombophilic defects were equally distributed, excepted for high FVIII levels (48.1% vs 34.9%; $p < 0.001$). Fetal loss rates were lower in women with high TAFI levels. Total fetal loss rates were 22.5% vs 27.9% (odds ratio 0.72; 95% CI, 0.49-1.07); early fetal loss rates 19.2% vs 24.9% (0.71; 0.48-1.05); total recurrent fetal loss rates 3.8% vs 7.9% (0.43; 0.19-0.96); and recurrent early fetal loss rates 2.8% vs 7.0% (0.38; 0.15-0.92). Our data provides evidence that high TAFI levels may protect against early and recurrent early fetal loss in women from families with thrombophilia.

Trombin-activatable fibrinolysis inhibitor (TAFI) is a procarboxypeptidase which suppresses fibrinolysis by removing carboxy-terminal lysine residues from partially degraded fibrin.[1] These residues are involved in binding of plasminogen and tissue-type plasminogen activator, and in plasmin formation. TAFI is activated by thrombin, mainly in complex with thrombomodulin, and by plasmin. TAFI inhibits tissue plasminogen activator-induced fibrinolysis.[2] High TAFI levels might enhance the development of thrombosis, and consequently fetal loss as result of placental thrombosis. Recently, we demonstrated that high TAFI levels are not associated with an increased risk of fetal loss.[3] On the contrary, our data suggested a decline of this risk at increasing TAFI levels. We hypothesized that high TAFI levels during normal pregnancy protect against fetal loss.[4,5] This effect might be more pronounced in pregnant women who are at increased risk of fetal loss due to thrombophilic defects. Here we present the results of an additional analysis of data from our previously reported study, to test this hypothesis.

The study population contained female subjects from four pooled retrospective family cohort studies.[6-9] These studies were designed to estimate the absolute risk of venous thromboembolism (VTE), associated with either hereditary deficiencies of antithrombin, protein C or protein S, the prothrombin 20210A mutation, elevated factor VIII:C levels, or hyperhomocysteinemia. Probands in each of these studies were consecutive patients with documented VTE or premature atherosclerosis (age < 50 years), and one of these thrombophilic defects. Relatives, who were 15 years of age or older were identified by pedigree analysis and were enrolled after informed consent was obtained. The studies were approved by the institutional review boards of the participating hospitals. In addition to above mentioned thrombophilic defects, subjects were tested for factor V Leiden, and TAFI activity was measured. Applied assays have been described elsewhere.[3] Detailed information on obstetric history was obtained, using a questionnaire and reviewing medical records. Clinical data was collected prior to blood sampling. Women were evaluable if they had been pregnant before the end of the study. Women with only terminated or ectopic pregnancies were excluded from analysis.

Fetal loss was defined as early fetal loss if it had occurred within 22 weeks of gestation, or as late fetal loss after more than 22 weeks of gestation, according to the criteria of the World Health Organisation.[10] Recurrent fetal loss was defined as 2 or more fetal losses. Fetal loss rates were expressed as percentages of women with fetal loss. Fetal loss rates in women with TAFI levels above the 75th percentile (i.e. $\geq$ 115 IU/dl) were compared to those in women with lower levels. Pregnancies after prior VTE were excluded from analysis, considering that thromboprophylaxis might have influenced the outcome of these pregnancies. Differences between groups were evaluated by the student t test or Mann-Whitney U test for continuous data, and by Fisher exact test for categorical data. Odds ratios were adjusted for family clustering by random effects logistic regression. A two-tailed p-value of less than 0.05 was considered to indicate statistical significance. Statistical analyses were performed using SAS software, version 9.1. (SAS-institute inc., Cary, North Carolina, USA)

The study cohort contained 1557 women, both probands and relatives. Of these women, 175 were excluded because they were younger than 15 years of age, had deceased or did not consent; 409 who

had never been pregnant or had had only terminated pregnancies; 35 who had experienced VTE prior to their first pregnancy; and 95 because of missing TAFI measurements. The remaining 843 women were analyzed; 213 had high TAFI levels.

Their characteristics are summarized in Table 1. Age at time of first pregnancy was comparable in women with high TAFI levels and women with normal TAFI levels. Venous thromboembolism had occurred in 13 women with high TAFI levels (6.1%) and in 50 women with normal TAFI levels (7.9%; p=0.38). Thrombophilic defects were equally distributed among both groups, excepted high factor VIII levels that were more prevalent in women with high TAFI levels. Total numbers of pregnancies were 608 in women with high TAFI levels (median 3) and 1864 in women with normal TAFI levels (median 3). The number of early fetal losses per woman ranged from 1 to 8 in women with normal TAFI levels (median 1), and from 1 to 4 in women with high TAFI levels (median 1), respectively.

Table 1: Characteristics and fetal loss rates in women of the study population

	High TAFI levels (n=213)	Normal TAFI levels (n=630)	OR * (95% CI interval)	p
Age at 1st pregnancy, median (range), yr	24 (17-40)	25 (11-42)		0.14
VTE, n (%)	13 (6.1)	50 (7.9)		0.38
TAFI levels, U/dL, mean (SD)	127 (11)	96 (11)		
Pregnancies, n	608	1864		
Thrombophilic defects, % (n tested)				
Genetic defect				
Antithrombin deficiency	2.4 (209)	1.8 (625)		0.56
Protein C deficiency	2.8 (211)	4.0 (627)		0.53
Protein S deficiency type I	4.9 (206)	3.9 (615)		0.55
Prothrombin G20210A	10.9 (212)	13.0 (625)		0.47
Factor V Leiden	12.7 (212)	11.7 (625)		0.71
Any thrombophilic defect	30.2 (205)	30.9 (611)		0.93
Acquired/ genetic defect				
Factor VIII:C > 150 IU/dL	48.1 (206)	34.9 (619)		<0.001
Total fetal loss, n (%)	48 (22.5)	176 (27.9)	0.72 (0.49 -1.07)	0.128
Early	41 (19.2)	156 (24.9)	0.71 (0.48-1.05)	0.093
Late	9 (4.2)	22 (3.5)	1.18 (0.52-2.66)	0.674
Total recurrent fetal loss, n (%)	8 (3.8)	50 (7.9)	0.43 (0.19-0.96)	0.041
Early	6 (2.8)	44 (7.0)	0.38 (0.15-0.92)	0.028
Late	0 (0)	2 (0.3)	-	1.00

*Adjusted for family clustering

Of women with high TAFI levels, 48 experienced any fetal loss (22.5%), i.e. early or late fetal loss, compared to 176 women with normal TAFI levels (27.9%; p=0.128). Early fetal loss was observed less frequently in women with high TAFI levels than in women with normal TAFI levels (19.2% versus 24.9%; p=0.093). Six women (2.8%) versus 44 women (7.0%) had experienced recurrent early fetal loss (p=0.028). Recurrent late fetal loss was observed in 0 versus 2 women (0.3%). Lower recurrent fetal loss rates in women with high TAFI levels versus women with normal TAFI levels were observed in subgroups of women with established persistent thrombophilic defects (i.e. hereditary deficiencies of antithrombin, protein C or protein S, factor V Leiden, and prothrombin G20210A mutation) and women without these defects, respectively (Table 2). Differences in fetal loss rates were similar, comparing women with high factor VIII levels to women with normal factor VIII levels, and considering that these levels were measured at enrolment (i.e. end of follow-up) and therefore might be different from factor VIII levels at time of pregnancy (Table 3). Differences in these subgroup analyses were not statistically significant. Of 35 women who were excluded from analysis, because they had had VTE prior to their first pregnancy, 29 women had any thrombophilic defect. None of 4 excluded women with high TAFI levels had recurrent fetal loss, compared to 1 of 31 women (3.2%) with normal TAFI levels.

Table 2: Fetal loss rates in women with thrombophilic defects

Women	Any thrombophilic defect* (n=251)		No thrombophilic defect (n=565)	
	High TAFI (n=62)	Normal TAFI (n=189)	High TAFI (n=143)	Normal TAFI (n=422)
Total recurrent fetal loss, n (%)	3 (4.8)	14 (7.4)	5 (3.5)	35 (8.3)
Early	2 (3.2)	11 (5.8)	4 (2.8)	32 (7.6)
Late	0 (0)	1 (0.5)	0 (0)	1 (0.2)

* Hereditary deficiency of antithrombin, protein C or protein S, factor V Leiden or prothrombin G20210A

Table 3: Fetal loss rates in women with high Factor VIII levels

Women	High Factor VIII (n=315)		Normal Factor VIII (n=510)	
	High TAFI (n=99)	Normal TAFI (n=216)	High TAFI (n=107)	Normal TAFI (n=403)
Total recurrent fetal loss, n (%)	4 (4.0)	13 (6.0)	3 (2.8)	34 (8.4)
Early	3 (3.0)	10 (4.6)	2 (1.9)	31 (7.7)
Late	0 (0)	1 (0.5)	0 (0.0)	1 (0.25)

This study showed a lower risk of fetal loss in women with high TAFI levels, compared to women with normal TAFI levels. Overall, the risk of fetal loss was declined with 28%, mainly due to a 29% risk reduction in early fetal loss. This apparently protective effect of high TAFI levels was most

pronounced for early recurrent fetal loss (risk reduction 62%). Our findings are in agreement with the only previous study on this issue.[11] That study addressed the association between single nucleotide polymorphisms (SNPs) and recurrent fetal loss in 86 women with recurrent fetal loss and in 72 controls with uncomplicated pregnancies. Two SNPs (+505A/A and + 1583 A/A) were associated with increased TAFI levels and a lower risk of recurrent fetal loss. On the other hand, the +505 G/G polymorphism, leading to lower TAFI levels, was more frequently demonstrated in women with recurrent fetal loss.[11]

Experiments in mice provided evidence that fibrin degradation products may induce apoptosis of trophoblasts, resulting in fetal loss.[12] Inhibition of fibrinolysis by TAFI is a possible explanation for the lower fetal loss rate in women with high TAFI levels in our study. If the protective effect of high TAFI levels is due to less apoptosis of trophoblasts, it will especially reduce the risk of early fetal loss, as we demonstrated. Considering that thrombophilic defects are associated with an increased risk of fetal loss,[13-15] we speculated that this might be due to the generation of more thrombin and consequently, more fibrin and fibrin degeneration products in pregnant women with thrombophilic defects, compared to pregnant women without these defects. However, we observed no difference in recurrent fetal loss rates between women with thrombophilic defects and women without a defect, though the protective effect of high TAFI levels was consistently observed in these subgroups. By exclusion of pregnancies after prior VTE, overall recurrent fetal loss rates may have been reduced, especially in women with thrombophilic defects, who are at higher risk of VTE. It is likely that anticoagulant thromboprophylaxis during pregnancies after prior VTE, also decreases thrombin generation and levels of fibrin degeneration products. It should be emphasized that these subgroup analyses were not powered to demonstrate differences in fetal loss rates.

Our observations are of clinical interest, because they suggest that another mechanism than placental thrombosis is involved in fetal loss. Thereby, a new perspective is opened for anticoagulant treatment in the prevention of early and late fetal loss.

Some limitations of this study warrant comments. First, selection bias may have been introduced by excluding pregnancies after prior VTE. As mentioned before, this would have declined the benefit of high TAFI levels, especially in women with thrombophilic defects, who may be at increased risk of both VTE and fetal loss. Second, although a systematic search for other causes of early and late fetal loss was not performed, due to the retrospective design of our study, it is likely that these were equally distributed. Finally, recall bias regarding fetal loss may have been introduced by the retrospective study design, but probably remained limited as clinical data was collected prior to thrombophilia testing and measurements of TAFI levels.

In conclusion, our data provides evidence that high TAFI levels may protect against early and recurrent early fetal loss.

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General discussion and future perspectives

Part I Menorrhagia and bleeding disorders

Part 1 of this thesis described that the frequency of an undiagnosed bleeding disorder, such as von Willebrand`s disease (VWD), in women with menorrhagia suggests that haemostatic testing should be added in the diagnostic work-up, prior to surgical intervention. Menorrhagia is often the first clinical manifestation in women with bleeding disorders, frequently beginning at menarche. Therefore, many affected patients initially visit their gynaecologist. A recent survey in the UK found that only 4% of the gynaecologists routinely considered VWD in the differential diagnosis in women of reproductive age.[1] We found in our clinic similar results. In the evaluation of the diagnostic work-up in women with menorrhagia in only 2 of 112 (2%) patients with menorrhagia a haemostatic evaluation was performed. Failure to diagnose bleeding disorders, such as VWD, may result in anaemia, impaired quality of life during menstruation, and unnecessary surgical interventions. Another important point of diagnosing an underlying bleeding disorder is that most of these disorders like VWD are inherited disorders, which could lead to screening of family members when a case is identified.

Until now, most studies excluded women with gynaecological abnormalities for the evaluation of the prevalence of underlying bleeding disorders.[2,3] However, in our study we found just as often haemostatic abnormalities in women with gynaecological abnormalities. These uterine abnormalities as fibroids may unmask a bleeding tendency. Hence haemostatic testing should also be considered in these cases. In our cohort of women with menorrhagia we could not identify a specific or combination of non-menorrhagia bleeding symptoms as a predictor for an underlying bleeding disorder. On the other hand, Philipp et al developed a screening tool to select women with menorrhagia for haemostatic testing.[4] However, they validated their screening tool in a population with a high prevalence of 71% of coagulation disorders.[5] This remarkably high prevalence limits the generalizability of their tool in another population.[6] Studies in the general population suggested that symptoms, like bleeding after tooth extraction, post-operative bleeding and postpartum bleeding, may be valuable to predict an inherited bleeding disorder.[7] In our cohort these non-menorrhagia bleeding symptoms did not predict for an underlying bleeding disorder. We suggest therefore that clinicians have to consider haemostatic evaluation in patients with menorrhagia, independently of the presence or absence of other bleeding symptoms or gynaecological abnormalities.

VWD is the most common bleeding disorder in the general population, but testing for other haemostatic disorders than VWD should also be considered. As we showed, women with menorrhagia, with and without gynaecological abnormalities have also an increased prevalence of low FXI levels or platelet defects.

Although 5-10% of the women of the general population have menorrhagia, there is no consensus on optimal management, even not for women with VWD. The goal of treatment is to improve quality of life by reducing the amount of blood loss during menstruation. Medical therapies for menorrhagia should be the first choice, including oral contraceptives, desmopressin, and antifibrinolytic agents, alone or in combination. However, it is not well studied what the benefits are of one formulation or

dosing strategy compared with another in the reduction of heavy menstrual bleeding especially in women with bleeding disorders. Kouides et al[8] suggest that both DDAVP and tranexamic acid reduce the menstrual blood loss in women with bleeding disorders. When medical therapies fail, surgical options as endometrial ablation and hysterectomy are possible suitable options/choices when fertility is no longer desired. Until comparative studies become available, treatment should be based on the bleeding disorder, gynaecological abnormalities, age, contraceptive needs, reproductive plans and personal preference of the individual patient.

Part II Bleeding issues in obstetrics

Part 2 of this thesis mainly described the clinical concerns and side-effects of the use of anticoagulants during pregnancy. Women with a history of a venous thrombo-embolism (VTE) have an increased risk for a recurrent VTE during pregnancy.[9, 10] These women have to use anticoagulants throughout the pregnancy and puerperium. This means that they have to inject themselves daily for about 10 months. Many of these women develop an aversion to these injections, often because of the hypersensitivity skin reactions. As we reported in our cohort of women who used LMWH during pregnancy, almost half of them developed hypersensitivity skin reactions during the use of nadroparin and had to switch to another preparation. Secondly, women on therapeutic dose of LMWH during pregnancy had an increased risk of PPH during vaginal and emergency caesarean section (CS) delivery. Taking these side-effects into account, it is important for each woman to make a good individual balance between the risk of VTE and the burden associated with anticoagulant therapy. Probably, a prophylactic or low dose instead of a therapeutic dose of anticoagulation during pregnancy might result in more net benefit in a subgroup of patients. The ACCP guidelines provide recommendations for VTE prophylaxis with LMWH for women who have an increased risk of VTE. However, these guidelines are mostly based on data from observational cohort studies in which the dosages of VTE prophylaxis were diverse and comparative groups were mostly lacking. [10-12] The optimal management for these patients is unknown and the guidelines offer different options. Therefore, it is not surprising that local hospitals use different treatment protocols. Consequently, it is important to have a treatment protocol in the local hospitals with good collaboration between gynaecologists and haematologists to improve patient care.

Implications for future research

The investigations described in this thesis support that bleeding disorders play an important role in the aetiology of women with menorrhagia. A novel finding of Chapter 3 is that women with menorrhagia have a significantly longer aPTT in patients compared to controls, apparently due to lower levels FXI in women with menorrhagia. FXI is a coagulation protein essential to normal haemostasis,

which acts by cleaving coagulation factor IX. Women with low levels FXI (<70%) are prone to excessive bleeding during menstruation. Bleeding manifestations do not correlate well with the plasma levels of FXI activity and bleeding episodes can vary widely among patients with similar FXI levels. Recent studies suggest that the incidence of mild FXI deficiency in Caucasians may be higher than expected. Until now, more than 90 FXI gene mutations, associated with a FXI deficiency have been reported. Most of these mutations are found in the Jewish population, which has a relatively high prevalence of FXI deficiency. The presence of mutations in FXI gene in women with menorrhagia could probably lead to lower levels of FXI. In the future, we want to investigate the prevalence of FXI gene mutations or polymorphisms in women with menorrhagia and a control group of healthy female volunteers matched by age with normal menstrual blood loss without hormonal treatment.

Another important function of FXI activation is to reduce fibrinolysis by promoting activation of thrombin activatable fibrinolytic inhibitor (TAFI). Loss or reduction of this normal pathway of inhibition in FXI deficiency fits well with the observation that FXI deficient individuals are prone to excessive bleeding after surgery or injuries to areas with high levels of fibrinolysis. But some individuals could have multiple defects, probably also in the fibrinolytic pathway. Therefore, it is conceivable that women with low levels of TAFI and FXI could have an increased bleeding tendency such as menorrhagia. In the future we want to investigate the role of the different fibrinolytic factors in women with menorrhagia.

Because haemostatic testing is expensive and impractical to routinely perform in gynaecological practice, it would be useful to develop a screening tool to select women with a high a priori risk of a bleeding disorder. In our cohort, the subgroups of patients are now too small to reach statistical significance, therefore we want to enlarge our cohort of women with menorrhagia. Second, it would be useful to standardize the treatment of women with menorrhagia, especially in women with bleeding disorders. Therefore, we need more comparative studies to evaluate the different treatment options.

Given the absence of studies about the optimal dosage of LMWH as thromboprophylaxis for women with an increased risk of VTE, a comparative trial of two different dosages of LMWH will improve patient care. Consequently, this will lead to more standardization in the treatment protocols of the different local hospitals and consequently national and international protocols. The main outcome of such a trial should be the effectiveness of the anticoagulation and the risk of VTE. The second outcomes should be the risk of side-effects like PPH and hypersensitivity skin reactions. Accordingly, the individual risk of side-effects as PPH and allergic skin reactions should be balanced with the risk of VTE. Maybe in a subgroup of patients a prophylactic dose of anticoagulation during pregnancy might result in more net benefit.

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Summary

Part I Menorrhagia and bleeding disorders

Menorrhagia is a common gynaecologic problem in women of reproductive age, accounting for a considerable proportion of gynaecology referrals. Although a variety of gynaecologic, endocrine, or other systemic causes may be responsible, an underlying aetiology is identified in only 50% of cases.[1] Underlying bleeding disorders may play a role in women with menorrhagia.

Chapter 1 summarized the evidence for timing of haemostatic testing during the menstrual cycle in women with a suspected bleeding disorder. Studies that determined the cyclic variation of platelet function, von Willebrand factor, factor VIII, factor IX, factor XI, factor XIII, d-dimer, PAI-I, tPA, alpha-2-antiplasmin and fibrinogen during normal menstrual cycle without hormonal contraceptives were systematically reviewed. In total, 1046 studies were identified of which 30 studies (25 longitudinal and 5 cross-sectional studies) were included. Overall, most of the studies found no cyclic variation in von Willebrand factor, FVIII, FXI, FXIII, fibrinolytic factors (PAI, t-PA, uPA, d-dimer and α2-antiplasmin) and fibrinogen. However, in studies where these variables showed any variation, they reached the lowest levels during menstrual and early follicular phase. This was most evident for von Willebrand, Factor FVIII and platelet function tests. We concluded that the most sensitive timing for haemostatic testing during menstrual cycle seems to be menstrual and early follicular phase.

Chapter 2 reported the work-up of menorrhagia in routine gynaecological practice, with a special interest in haemostatic evaluation, and the outcome of individualized treatment in our centre. A retrospective medical chart review of consecutive patients referred for menorrhagia to the University Medical Centre of Groningen between January 2006 and January 2007 was performed. In April 2008, all women were contacted for a structured telephone interview evaluating the effectiveness of their therapy. In total, 112 patients were included, with a median age of 42 years. Twenty-nine percent were anaemic (hemoglobin <12.0 g/dL). Seventy-one (63%) had unexplained menorrhagia. Only two patients had haemostatic evaluation; Von Willebrand's disease was excluded in both. Forty percent (29/71) needed two or more different therapies, 17% (12/71) needed three different therapies and two patients needed a total of seven different therapies. Eight patients underwent a hysterectomy, six of them after endometrial ablation. Most patients (80%) were successfully treated medically or surgically and were satisfied with their therapy during follow-up. Eleven patients declined therapy and accepted their heavy periods. In conclusion, haemostatic evaluation in women with unexplained menorrhagia is uncommon in gynaecological practice in our centre. Although most of the patients were satisfied with their treatment, a significant number required hysterectomy and another important proportion had to accept their menorrhagia. Therefore we hypothesized that the identification of haemostatic disorders might improve care for these women.

To follow up on these findings, **Chapter 3** addressed the prevalence of bleeding disorders and symptoms in women with menorrhagia, with and without gynaecological abnormalities. Hundred-and-two consecutive patients referred for menorrhagia between March 2007 and December 2010

were included. All underwent gynaecologic evaluation. Menorrhagia was confirmed by a pictorial bleeding assessment chart score above 100. Patients and controls (28 healthy volunteers without menorrhagia) had haemostatic testing in the 1st week after menstruation. Forty-six percent of patients were anaemic, 61% had low ferritin. Twenty-six percent of patients had endometrial polyps or submucosal uterine myoma, sufficient to explain menorrhagia. An underlying bleeding disorder was found in 29% vs 11% (p=0.04) of the patients vs controls, and in 31% vs 27% of the women with unexplained vs explained menorrhagia (p=0.75). We diagnosed 6 cases of VWD, 4 cases of FXI deficiency and one FVII deficiency. The only abnormalities found in controls were platelet aggregation defects (11% vs 23% in patients). Patients had a significantly longer aPTT compared to controls (26.5 vs 25.0 sec; p=0.001) caused by lower median levels of FXI (100 vs 124 IU/dL; p<0.001). Although non-menorrhagia bleeding symptoms were more prevalent in patients than in controls, additional bleeding symptoms did not predict for an underlying bleeding disorder. In conclusion, bleeding disorders play an equally important role in the aetiology of menorrhagia, in women with and without gynaecological abnormalities. A novel finding is the occurrence of low, but not deficient levels of factor XI.

In **chapter 4** the gynaecological and obstetrical symptoms in an unselected cohort of women with moderate and severe VWD in the Netherlands were assessed, as part of the nation-wide 'Willebrand in the Netherlands (WiN) study. 423 women aged ≥16 years were included. Bleeding severity was measured using the Tosetto Bleeding Score (BS). Menorrhagia, defined as occurrence of ≥2 menorrhagia symptoms, was reported by 81%. Of all VWD women, 78% received any kind of treatment for menorrhagia and 20% underwent a hysterectomy predominantly because of severe menstrual bleeding. Over half of the women reported more blood loss than expected with a normal delivery. In 52% of reported pregnancy losses curettage was needed because of bleeding. Mean number of live births was 1.9, which is comparable with the general Dutch population. We concluded that women with moderate or severe VWD frequently have menorrhagia in need of treatment, with 20% of the VWD women undergoing a hysterectomy. Bleeding complications occurred in over 50% of the women after childbirth or pregnancy loss. Progeny seems not to be affected in women with moderate or severe VWD.

Part II Bleeding issues in obstetrics

Low-molecular-weight heparins (LMWH) are the most commonly used anticoagulant during pregnancy for the treatment and prevention of venous thrombo-embolism (VTE). However, the size of the associated risk of postpartum haemorrhage (PPH) is unknown. Moreover, hypersensitivity skin reactions due to the use of LMWH are frequently seen, but are probably underreported in the literature. Chapter 5 and 6 addressed these complications of LMWHs during pregnancy. **Chapter 5** assessed the bleeding risk of therapeutic dosage of LMWH, also in relation to time between last dose LMWH and delivery. In this study, we prospectively followed 88 pregnant women between 1999 and 2009 who used therapeutic anticoagulation. Controls were pregnant women without LMWH, matched 1:4 for

parity, mode of delivery, age, gestational age and delivery date. PPH was defined as >500 ml blood loss for vaginal delivery (severe PPH in vaginal delivery as >1000 ml) and >1000 ml for caesarean section (CS). Women were divided into subgroups by the interval between last dose of LMWH and delivery (<12, 12-24hrs, >24hrs). Risk of PPH after vaginal delivery was 30% and 18% for LMWH-users and non-users, respectively (OR 1.9, 95%CI 1.1-3.5). Risk of severe PPH after vaginal delivery was not different (5.6 vs 5.0%; OR 1.1; 0.4-3.6). Risk of PPH after CS was 12% in LMWH-users and 4% in non-users (OR 2.9; 0.5-19.4). Both events of LMWH-users occurred after emergency CS. The risk of PPH associated with delivery within 24 hours after last dose of LMWH was 1.2 fold higher (95%CI 0.4-3.6) compared to a larger interval. We concluded that therapeutic dosage of LMWH carries an increased risk of PPH after vaginal delivery. The interval between last dose of LMWH and delivery does not influence the risk of PPH. **Chapter 6** evaluated, in the same cohort, the incidence of hypersensitivity skin reactions due to the use of LMWH in pregnancy, and the subsequent management of anticoagulation. All women were started on nadroparin and switched to another preparation when hypersensitivity skin reactions occurred. We included 135 pregnancies in 88 women. Overall, in 52 of 135 pregnancies (39%), women switched at least once to another anticoagulant due to the development of hypersensitivity skin reactions. Switching to another preparation of LMWH was effective in 77% of the cases. In 23% of the cases skin reactions recurred and another switch had to be made. In a subgroup of women, it is necessary to ultimately switch to VKA or fondaparinux. **Chapter 7** describes ten patients who used fondaparinux during 12 pregnancies between 2003 and 2010 because of hypersensitivity skin reactions. All women were followed prospectively. They initially used LMWH but developed hypersensitivity skin reactions. Two patients used fondaparinux during two pregnancies, both started in the 1st trimester of the 2nd pregnancy. In all other pregnancies, fondaparinux was started in the 2nd or 3rd trimester. Fondaparinux was not associated with skin reactions or other side-effects. None of the 13 infants had congenital abnormalities or neonatal bleeding. Concluding, fondaparinux seems to be an alternative treatment for women who had allergic skin reactions to LMWH. Given the limited data, we do not recommend the use of fondaparinux in the 1st trimester.

Chapter 8 evaluated the reproductive choices and obstetrical experiences in the current generation of carriers of haemophilia in our Haemophilia Centre in the north of the Netherlands, a largely secular country with liberal abortion laws and a unique tradition of home births. We performed a retrospective survey among haemophilia carriers. We sent a questionnaire to 74 carriers of whom 65 were available, and 75% responded. Median age was 41 (range 20–83) years. Of the 49 women, 46 experienced 120 pregnancies: 25 resulted in fetal loss, two in pregnancy termination (one for haemophilia) and 93 in live births. No woman had chosen not to start a family. Mean number of children was 2.0, 2.4 vs. 1.8 in women with and without sons with haemophilia (P = 0.008), respectively. Twenty women (20 of 46) were unaware of their carriership during 1st pregnancy; they were younger at 1st pregnancy than known carriers (25 vs 29 years, P = 0.03). Twenty-three percent reported bleeding complications during the first delivery. Overall, 10% vs. 3% of deliveries were complicated by a primary and secondary PPH, respectively. In our Haemophilia Centre, carrier state has not influenced reproductive choices in the

past, other than older age at first pregnancy. Carriers of haemophilia have an increased risk of primary PPH.

Thrombin-activatable fibrinolysis inhibitor (TAFI) is a procarboxypeptidase, activated by thrombin, mainly in complex with thrombomodulin, and by plasmin that inhibits tissue plasminogen activator-induced fibrinolysis. Through this inhibition of fibrinolysis, high TAFI levels might enhance the development of thrombosis, and consequently placental thrombosis, which can be a cause of fetal loss. On the other hand, fetal loss may be also induced by apoptosis of trophoblasts due to high levels of fibrin degradation products. Consequently, high TAFI levels, which inhibits fibrinolysis and gives accordingly less fibrin degradation products, could also reduce this risk, especially in women with thrombophilia who generate more thrombin. In **chapter 9** this hypothesis was tested in women from 4 family cohorts, originally designed to estimate the risk of venous thromboembolism, associated with either hereditary deficiencies of antithrombin, protein C or protein S, prothrombin 20210A, factor V Leiden, elevated factor VIII levels or hyperhomocysteinemia. In these women, the fetal loss rate was retrospectively assessed. We compared women with high TAFI levels (> 75th percentile, i.e. 115 U/dl) to women with normal TAFI levels. Of 1557 women, 843 (probands and relatives) were evaluable of whom 213 had high TAFI levels. Women with high TAFI levels and women with normal TAFI levels were comparable for age at time of first pregnancy (24 versus 24 years) and mean number of pregnancies (2.9 versus 3.0). Thrombophilic defects were equally distributed, excepted for high FVIII levels (48.1% versus 34.9%; $p < 0.001$). Fetal loss rates were lower in women with high TAFI levels. Total fetal loss rates were 22.5% vs 27.9% (odds ratio 0.72; 95 % CI, 0.49-1.07); early fetal loss rates 19.2% vs 24.9% (0.71; 0.48-1.05); total recurrent fetal loss rates 3.8% vs 7.9% (0.43; 0.19-0.96); and recurrent early fetal loss rates 2.8% vs 7.0% (0.38; 0.15-0.92). Our data provides evidence that high TAFI levels may protect against early and recurrent early fetal loss in women from families with thrombophilia.

Samenvatting
(voor de niet-medicus)

Dit proefschrift gaat over stollingsstoornissen bij verschillende problemen in de gynaecologie en verloskunde. Ik heb zowel naar verhoogde bloedingsneiging, als naar problemen van trombose gekeken. De verschillende studies werden uitgevoerd bij vrouwen met menstruatieproblemen, vrouwen die bloedverdunners gebruikten tijdens de zwangerschap, vrouwen die zelf een bloedingsziekte hadden of die dit door konden geven aan hun kinderen en vrouwen met herhaalde miskramen. Met de resultaten van dit proefschrift kunnen patiënten beter geïnformeerd worden en in een aantal situaties ook beter behandeld worden.

Deel 1 Menorragie en stollingsstoornisssen

In deel 1 van dit proefschrift worden de verschillende aspecten van menorragisch bloedverlies en stollingsstoornissen beschreven. Menorragisch bloedverlies is hevig menstrueel bloedverlies, overeenkomend met meer dan 80 ml bloedverlies per menstruele cyclus, wat o.a. kan leiden tot bloedarmoede en een verminderde kwaliteit van leven. Ongeveer 10% van de vrouwen heeft last van hevige menstruaties, een deel van deze vrouwen wordt tenminste eenmaal door de huisarts voor een consult naar de gynaecoloog verwezen. Er zijn vele mogelijke oorzaken van hevig menstrueel bloedverlies, zoals een verstoring van de hormoonbalans, myomen (vleesbomen) en poliepen in de baarmoeder. De helft van de gevallen wordt echter maar verklaard door één van deze afwijkingen. Een andere oorzaak die in de laatste decennia steeds vaker gediagnosticeerd wordt zijn onderliggende bloedstollingsstoornissen. Bloedstolling is een buitengewoon complex proces waarbij vooral de bloedplaatjes en een groot aantal eiwitten in het bloed, de zogenaamde stollingsfactoren, betrokken zijn. Falen van de bloedstolling leidt tot een verhoogde bloedingsneiging, waarbij mensen langer bloeden dan normaal.

Onderliggende bloedstollingsstoornissen die tot nu toe beschreven zijn bij vrouwen met een hevige menstruatie zijn o.a. de ziekte van Von Willebrand, plaatjesaggregatiestoornissen en een verlaagde waarde van één van de stollingsfactoren. De ziekte van Von Willebrand is de meest voorkomende erfelijke bloedingsziekte. In de algemene populatie is 1 op de 1000 individuen aangedaan. Van deze erfelijke afwijking in de bloedstolling bestaan verschillende typen en de mate van ernst kan variëren. De oorzaak is een verlaagd gehalte aan het stollingseiwit Von Willebrand Factor (VWF) of een abnormaal functionerend VWF. De ziekte komt voornamelijk tot uiting door slijmvliesbloedingen (vooral neus- en tandvleesbloedingen). Er bestaat een verhoogd risico op nabloedingen bij het trekken van kiezen, operaties en trauma's. Bij vrouwelijke patiënten kan het leiden tot hevige bloedingen tijdens de menstruatie of na een bevalling. Een andere bloedstollingsstoornis is een plaatjesaggregatiestoornis. Hierbij is er een stoornis in het samenplakken van bloedplaatjes, wat nodig is om een goed stolsel te kunnen vormen. Een stoornis in deze onderlinge hechting kan leiden tot o.a. slijmvliesbloedingen, maar ook tot hevige menstruaties.

In **hoofdstuk 1** wordt een systematisch overzicht gegeven van alle relevante onderzoeken die in de afgelopen 30 jaren stollingsfactoren gedurende de natuurlijke menstruele cyclus hebben gemeten.

In totaal werden 1046 studies gevonden, waarvan uiteindelijk 30 studies relevant bleken te zijn. De meeste studies lieten geen verandering zien van de verschillende stollingsfactoren als Von Willebrand factor, factor VIII, factor XI, factor XIII, fibrinolytische factoren en fibrinogeen gedurende de menstruele cyclus. Enkele studies lieten wel een cyclische verandering zien. Deze studies lieten een verlaging van factor VIII en Von Willebrand factor zien gedurende de eerste helft van de menstruele cyclus, oftewel de eerste 2 weken na de eerste dag van de menstruatie. Concluderend lijkt de beste timing voor stollingsonderzoek tijdens de menstruele cyclus de eerste helft te zijn, omdat je dan de meeste kans hebt dat je een verlaagde waarde van een stollingsfactor vindt.

Eerder uitgevoerd onderzoek liet zien dat stollingsstoornissen mogelijk de oorzaak kunnen zijn van hevige menstruaties. Derhalve hebben wij in **hoofdstuk 2** bestudeerd hoe vaak er in de klinische praktijk gedacht wordt aan onderliggende stollingsstoornissen bij vrouwen met hevige menstruaties en hoe vaak aanvullend stollingsonderzoek wordt verricht. Wij hebben in 2007 terugkijkend alle patiënten met hevige menstruaties onderzocht die in 2006 werden verwezen naar de polikliniek gynaecologie van het UMCG. Er bleek bij slechts 2 van de 102 patiënten aanvullend stollingsonderzoek te zijn verricht, beide patiënten hadden geen stollingsstoornis. In april 2008 werden alle 102 patiënten telefonisch benaderd voor een interview over de ingestelde therapie. De meeste patiënten (80%) werden succesvol behandeld en waren tevreden met de ingestelde behandeling. Echter, 40% hiervan bleek twee of meer behandelingen nodig te hebben gehad. De baarmoeder werd bij 8 patiënten verwijderd, bij 6 patiënten werd dit verricht nadat het baarmoederslijmvlies al was verwijderd. Elf patiënten werden niet meer behandeld en accepteerden de hevige menstruaties. Concluderend is een groot deel van de patiënten tevreden met de ingestelde therapie, een deel echter pas nadat ze een baarmoederverwijdering hadden ondergaan. Een ander deel accepteerde de hevige menstruaties. Op basis van deze gegevens veronderstelden wij dat er ruimte was voor het optimaliseren van de behandeling voor deze patiënten, mogelijk door het identificeren van onderliggende stollingsstoornissen.

Daaropvolgend hebben wij een studie opgezet om het voorkomen van onderliggende stollingsstoornissen bij vrouwen met hevige menstruaties te onderzoeken. Daarvoor hebben wij in 2007 de M(enstruatie)-poli opgezet in het UMCG. Alle patiënten die doorverwezen werden met hevige menstruaties, kregen voorafgaand aan het polikliniekbezoek een vragenlijst thuis gestuurd met o.a. vragen over de menstruatie en symptomen die kunnen passen bij een verhoogde bloedingsneiging. Hierbij gaat het om symptomen als hevige menstruaties vanaf de eerste menstruatie (=menarche), voorkomen van bloedneuzen, nabloedingen na operaties of bevallingen. Tijdens het polikliniekbezoek werd bij al deze patiënten gynaecologisch onderzoek verricht naar onderliggende gynaecologische afwijkingen zoals vleesbomen of poliepen, die ook kunnen zorgen voor hevige menstruaties. In **hoofdstuk 3** worden de resultaten van deze studie beschreven. In totaal werden 102 patiënten geïncludeerd. Aanvullend werden ook 28 gezonde vrijwilligsters (= controlegroep) met normaal menstrueel bloedverlies gevraagd om mee te doen. Bij zowel de vrijwilligsters als de patiënten werd stollingsonderzoek verricht in de eerste week na de menstruatie. In 26% van de patiënten werd een onderliggende gynaecologische oorzaak (vleesboom of poliep) gevonden als oorzaak voor de

hevige menstruaties. Bij 46% van de patiënten bleek sprake van bloedarmoede. Een onderliggende stollingsstoornis werd bij 29% van de patiënten en 11% van de controles gevonden. We vonden bij 6 patiënten de ziekte van Von Willebrand (VWD), 4 patiënten hadden een verlaagde factor XI en één patiënt had een verlaagde factor VII spiegel. Bij de gezonde controles werden alleen stoornissen in de functie van de bloedplaatjes gevonden. Verder werd bij de patiënten een significant langere aptt (=stollingstijd) gevonden, waarschijnlijk doordat de patiënten gemiddeld een significant lagere FXI spiegel hadden. Het hebben van een verhoogde bloedingsneiging op basis van symptomen, bijv. bloedneuzen, bleek in onze patiëntengroep geen voorspeller te zijn voor een onderliggende stollingsstoornis. Concluderend spelen onderliggende stollingsstoornissen een belangrijke rol bij vrouwen met hevige menstruaties, dus adviseren wij bij deze patiënten een onderliggende stollingsstoornis overwogen moet worden als mogelijke oorzaak. Von Willebrand ziekte (VWD) is een erfelijke stollingsafwijking die even vaak voorkomt bij mannen als bij vrouwen. Echter bij vrouwen met VWD geeft de ziekte eerder klachten door vrouwspecifieke symptomen als hevig menstrueel bloedverlies en bloedingen bij de bevalling.

In **hoofdstuk 4** zijn de gynaecologische en obstetrische klachten bij vrouwen met matig-ernstige of ernstige VWD bestudeerd. Deze studie is een onderdeel van het grote Willebrand in Nederland (WiN) onderzoek. De studie omvatte 432 vrouwen, ruim 80% van hen had last (gehad) van hevige menstruaties. Bijna al deze vrouwen gebruikten of hebben eerder hormonale anticonceptiva (hormoon therapie, de pil of Mirena) gebruikt om de hoeveelheid menstrueel bloedverlies te doen verminderen. Tevens had 20% van de vrouwen een baarmoederverwijdering ondergaan en bij de vrouwen boven de 40 jaar was dit zelfs 28%. Een baarmoederverwijdering werd vaker gecompliceerd door een bloeding als de diagnose VWD ten tijde van de operatie nog niet bekend was. Ook daarom is het erg belangrijk dat gynaecologen onderliggende stollingsafwijkingen in overweging nemen bij vrouwen met menorragisch bloedverlies. Verder zijn er voor patiënten met een onderliggende stollingsstoornis andere aanvullende behandelopties beschikbaar die de bloedstolling activeren. Desmopressine en tranexaminezuur zijn medicamenten die de bloedstolling beïnvloeden waarmee de hoeveelheid bloedverlies tijdens de menstruatie verminderd. Verder had meer dan de helft van de vrouwen met VWD overmatig bloedverlies tijdens de bevalling. Dit werd gedefinieerd als meer dan de verwachte hoeveelheid bloedverlies. De vrouwen met VWD bleken ook vaker een bloedtransfusie nodig te hebben in vergelijking met de algemene populatie. Het gemiddeld aantal levendgeborenen was 1.9 per vrouw, dit is vergelijkbaar met de algemene populatie. Dit suggereert dat vrouwen met de ziekte van Von Willebrand, ondanks de bloedingsproblemen, net zo veel kinderen krijgen als andere vrouwen.

Deel 2 Bloedingsproblemen in de verloskunde

In deel 2 van dit proefschrift worden o.a. de verschillende complicaties van het gebruik van bloedverdunners in de vorm van laag moleculair gewicht heparines (LMWH) in de zwangerschap beschreven. LMWH is het middel van 1e keus bij zowel behandeling als preventie van veneuze trombo-

embolieën tijdens de zwangerschap. Het risico op een bloeding na de bevalling hierbij is echter niet goed bekend. Tevens hebben patiënten vaak last van allergische huidreacties als gevolg van het dagelijks spuiten van de LMWH.

In **hoofdstuk 5 en 6** beschrijven wij de complicaties van het gebruik van bloedverdunners tijdens de zwangerschap. In hoofdstuk 5 wordt het risico op een bloeding na de bevalling onderzocht bij vrouwen die tijdens de zwangerschap een hoge dosering bloedverdunners gebruikten. Verder onderzochten wij of het tijdstip waarop de laatste gift van de bloedverdunner was gegeven invloed had op de hoeveelheid bloedverlies. Voor dit onderzoek hebben wij alle vrouwen die tussen 1999 en 2009 in het UMCG bloedverdunners kregen tijdens de zwangerschap geïncludeerd. Deze vrouwen werden zowel op de afdeling verloskunde als ook op de afdeling stolling gezien. Daarnaast hebben we een controle groep geïncludeerd. Dit waren vrouwen die geen bloedverdunners gebruikten, maar die in dezelfde periode in het UMCG zijn bevallen. Een bloeding na de bevalling (PPH) werd gedefinieerd als meer dan 500 ml voor een vaginale bevalling en als meer dan 1000 ml voor een keizersnede. Een ernstige PPH werd gedefinieerd als meer dan 1000 ml na een vaginale bevalling. Het risico op een PPH bleek 1.9 hoger voor vrouwen die bloedverdunners gebruikten rond een vaginale bevalling. Het risico op een ernstige PPH na een vaginale bevalling bleek niet verhoogd te zijn tijdens het gebruik van bloedverdunners. Het risico op een PPH na een keizersnede was 12% in de groep die bloedverdunners gebruikten vergeleken met 4% in de controle groep. Alle bloedingen werden echter gerapporteerd in de groep die een spoed-keizersnede ondergingen. Het risico op een PPH was niet verder verhoogd indien patiënten binnen 24 uur na de laatste gift LMWH bevielen.

In **hoofdstuk 6** wordt in hetzelfde cohort de incidentie van allergische huidreacties als gevolg van het gebruik van bloedverdunners tijdens de zwangerschap bestudeerd. Alle vrouwen startten met nadroparine, een bloedverdunner in de vorm van een LMWH, en switchten naar een ander middel als zij een allergische huidreactie ontwikkelden. We includeerden hiervoor 135 zwangerschappen van 88 vrouwen. In 52 van de 135 zwangerschappen (39%) moesten de vrouwen tenminste een keer switchen naar een ander preparaat i.vm. een allergische huidreactie. Het switchen was effectief in 77% van de gevallen. Derhalve concludeerden wij dat allergische huidreacties vaak voorkomen bij het gebruik van LMWH in de zwangerschap, maar het switchen naar een ander middel lijkt hierbij zinvol.

In **hoofdstuk 7** beschrijven we 12 zwangerschappen waarin fondaparinux als bloedverdunner werd gegeven in de zwangerschap. Fondaparinux is een alternatief antistollingsmiddel dat gebruikt werd indien patiënten allergische huidreacties hadden op meerdere LMWH preparaten. Er is nog weinig ervaring met dit middel bij zwangere vrouwen. Fondaparinux gaf in deze zwangerschappen geen allergische huidreacties. Ook werden geen aangeboren afwijkingen en bloedingen bij de pasgeborene gerapporteerd. Fondaparinux lijkt een goede alternatieve antistollingsbehandeling in de zwangerschap, echter gezien de beperkte ervaring adviseren wij nog steeds om dit middel niet te geven in de eerste drie maanden van de zwangerschap.

Hemofilie is een zeldzame geslachtsgebonden erfelijke bloedingsziekte. Vrouwen uit families waarin hemofilie voorkomt en die via de vrouwelijke lijn verwant zijn aan een hemofilie patiënt

hebben a priori een verhoogde kans om draagster te zijn. Draagsters kunnen een verhoogd risico op bloedingen hebben. Verder hebben zij 50% kans dat als zij een zoon krijgen, die zoon hemofilie heeft. Er zijn twee typen hemofilie. Bij hemofilie A is er sprake van een tekort aan stollingsfactor VIII en bij hemofilie B is er sprake van een tekort aan stollingsfactor IX. In **hoofdstuk 8** evalueren wij de verloskundige ervaringen in de huidige generatie draagsters hemofilie A en B. Hiervoor benaderden wij alle draagster hemofilie A en B (n=74) die bekend waren in onze kliniek, 75% van de draagsters respondeerden. De mediane leeftijd was 41 jaar. 46 van de 49 draagsters waren ooit zwanger geweest met in totaal 120 zwangerschappen. Het gemiddeld aantal kinderen was 2.0. De draagsters met een zoon met hemofilie hadden gemiddeld minder kinderen dan de draagsters zonder een kind met hemofilie (1.8 in vergelijking met 2.4). Twee vrouwen besloten om de zwangerschap af te breken, één vanwege hemofilie. In totaal bleken 20 draagsters tijdens de eerste zwangerschap niet op de hoogte te zijn van hun draagsterschap. Zij waren dan ook jonger tijdens hun eerste zwangerschap in vergelijking met draagsters die dit wel wisten. (25 in vergelijking met 29 jaar) In 23% van de gevallen werd de bevalling van het eerste kind gecompliceerd door een bloeding na de bevalling. Bij 10% van alle bevallingen trad de bloeding binnen de eerste 24 uur en in 3% meer dan 24 uur na de bevalling op. Wij concludeerden dat draagsters van hemofilie even veel kinderen lijken te krijgen als de algemene populatie, de leeftijd ten tijde van het eerste zwangerschap lijkt alleen hoger te zijn. Verder hebben draagsters een verhoogd risico op een bloeding na de bevalling.

Trombine-geactiveerde fibrinolyse remmer (TAFI) is een stollingseiwit dat de fibrinolyse remt. Fibrinolyse is het proces waarbij een bloedstolsel langzaam wordt afgebroken. Omdat TAFI de fibrinolyse remt, kan dit leiden tot trombose en daardoor ook tot trombose in de placenta wat weer kan leiden tot een miskraam. In **hoofdstuk 9** wordt het risico op (herhaalde) miskraam bij vrouwen met een hoge TAFI spiegel beschreven. Deze vrouwen zijn afkomstig uit een grote groep van families met een verhoogd trombose risico vanwege een erfelijke of verworven stollingsafwijking. Dit kon een deficiëntie van antitrombine, proteïne C of proteïne S, protrombine G20210A mutatie, factor V Leiden, hoge factor VIII spiegels of hyperhomocysteïnemie zijn. De miskramen werden terugkijkend onderzocht. We vergeleken vrouwen met een hoge TAFI spiegel (> 115 U/dl) met vrouwen met een normale TAFI spiegel. Er konden 843 vrouwen mee doen in deze studie. In totaal hadden 213 vrouwen een verhoogde TAFI spiegel. Het gemiddeld aantal zwangerschappen was vergelijkbaar in beide groepen. Echter het aantal miskramen was lager in de groep vrouwen met hoge TAFI spiegels (22.5 versus 27.9 %). Ook het aantal vrouwen met een herhaalde miskraam (>2) was significant lager in de groep vrouwen met een hoge TAFI spiegel (3.8 versus 7.9%). Concluderend, in tegenstelling met wat we hadden verwacht, beschermen hoge TAFI spiegels dus mogelijk tegen een vroege en een herhaalde vroege miskraam.

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List of publications

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Curriculum Vitae

Curriculum Vitae

Marieke Knol werd op 29 juli 1980 in Avereest geboren. In 1998 behaalde zij haar VWO diploma in Zwolle. Ditzelfde jaar startte zij, nadat zij uitgeloot was voor geneeskunde, met de studie MBRT (medische beeldvormende en radiotherapeutische technieken), waarin zij in 1999 haar propedeuse behaalde. In 1999 begon zij met de studie geneeskunde aan de rijksuniversiteit Groningen, waarna zij in 2005 het artsexamen behaalde. De wetenschappelijke stage werd o.l.v. dr. A. Hoek verricht op de afdeling Voortplantingsgeneeskunde van het UMCG. Haar co-schappen en keuze-coschap deed zij in het Martini ziekenhuis te Groningen. Na het behalen van het artsexamen in 2005 was zij achtereenvolgens werkzaam als ANIOS Obstetrie in het Leveste ziekenhuis te Emmen en als ANIOS algemene en oncologische gynaecologie in het Universitair Medisch Centrum Groningen. Van oktober 2007 tot en met juli 2011 was zij werkzaam op de afdeling Hemostase en Trombose van het Universitair Medisch Centrum Groningen als stollingsarts-onderzoeker. Tevens was zij in dit ziekenhuis werkzaam als arts-assistent Obstetrie en Gynaecologie. Gedurende deze periode werden onder leiding van prof. dr. J.C. Kluin-Nelemans en prof. dr. A.G.J. van der Zee de studies uitgevoerd, die in dit proefschrift zijn beschreven. Op 1 juli 2011 startte zij met de opleiding Obstetrie en Gynaecologie in het Cluster Groningen, allereerst in het Deventerziekenhuis (opleider dr. P.J.Q. van der Linden) en per 1 juli 2012 in het Universitair Medisch Centrum Groningen (opleider prof. dr. M.J.E. Mourits).